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ITHACA, NEW YORK
SEPTEMBER, 20-22, 1976



THIRD WORKSHOP ON FIRE BLIGHT RESEARCH

APPLE AND PEAR DISEASE WORKERS

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THIRD WORKSHOP

ON

FIRE BLIGHT RESEARCH

Ramada Inn

Ithaca, New York

September 20-22, 1976

Cornell University

New York State Agricultural Experiment Station

United States Department of Agriculture

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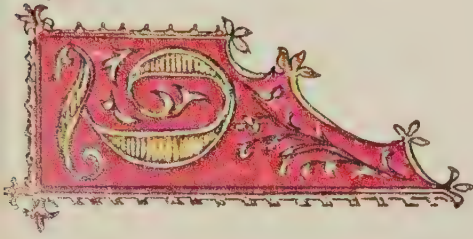
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he Trustees of The Cornell University, at Ithaca, in the State of New York, to all and to each to whom these Letters may come,

GRADUATES

BEFORE, The appropriate Faculties of the University have recommended to Us

Joseph Charles Arthur, B. S., M. S.

as having satisfactorily pursued the Studies and passed the Examinations required for that Degree: We, therefore, by virtue of the authority in Us vested. do hereby certify thereto, and confer on him the Degree of

Doctor of Science,

with all the Rights, Privileges, and Honors, here or elsewhere thereunto appertaining.

in Witness Whereof The Seal of the University and the Signature of the President thereof are hereunto affixed.



Given at ITHACA, on the Seventeenth Day of June, in the Year of our Lord One Thousand Eight Hundred and Eighty-six, of the Republic the One Hundred and Tenth, and of the University the Eighteenth.

C. K. Adams President.

In 1886, J. C. Arthur submitted the first doctoral dissertation on fire blight. After Burrill named the organism micrococcus amylovorus in 1880, Arthur was the first person to prove pathogenicity of the bacterium through the Koch's postulates on apple, pear, and other rosaceous host plants.

Reference: "Proof that bacteria are the direct cause of the disease in trees known as pear blight."
Amer. Assoc. Adv. Science Proc. 34:295-298, 1885.

Additional: Baker, K. F.
Fire blight of pome fruits: the genesis of the concept that bacteria can be pathogenic to plants.
Hilgardia 40:603-633, 1971.

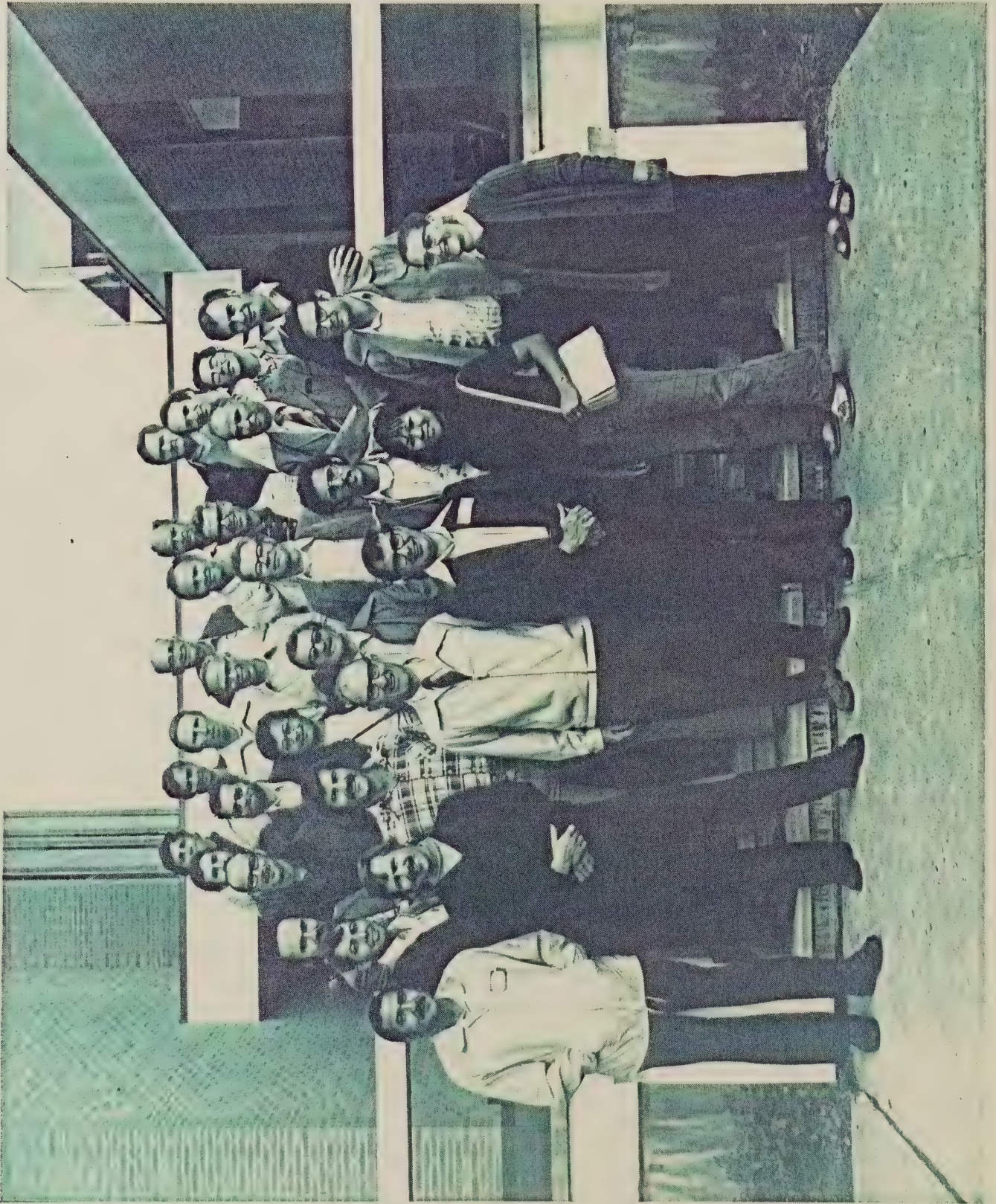
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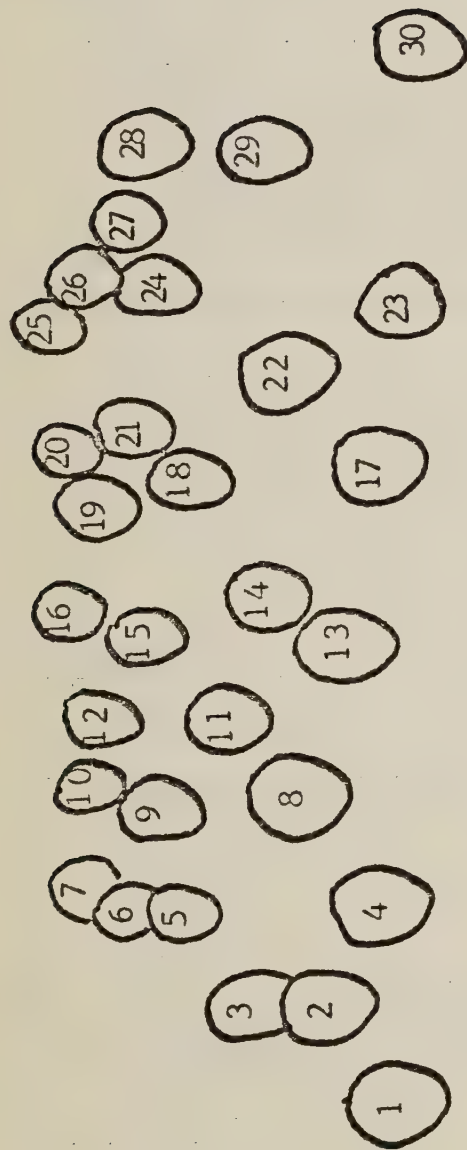
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Participants in the APDW 3rd Fire Blight Workshop held at Ithaca, N. Y.,
visit to New York State Agricultural Experiment Station, Geneva. Photo by G. Catlin

- | | | |
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| 2. P. C. Pecknold (Indiana) | 12. A. L. Jones (Michigan) | 22. D. F. Ritchie (Michigan) |
| 3. W. Zeller (Germany) | 13. E. K. Wade (Wisconsin) | 23. C. Helkie (Maryland) |
| 4. H. S. Aldwinckle (New York) | 14. L. J. Coulombe (Quebec) | 24. C. A. R. Meijneke (The Netherlands) |
| 5. S. M. Ries (Illinois) | 15. R. N. Goodman (Missouri) | 25. V. J. Carroll (New York) |
| 6. W. G. Bonn (Ontario) | 16. R. C. Blake (Ohio) | 26. R. C. Seem (New York) |
| 7. G. T. Berggren (Missouri) | 17. J. Kuc (Kentucky) | 27. S. V. Beer (New York) |
| 8. D. C. Opgenorth (Wisconsin) | 18. S. H. Davidson (Delaware) | 28. L. N. Gibbins (Ontario) |
| 9. A. G. Otterbacher (Illinois) | 19. M. N. Schroth (California) | 29. S. V. Thomson (California) |
| 10. A. K. Chatterjee (California) | 20. T. van der Zwet (Maryland) | 30. E. Billing (England) |

Workshop participants not present for the picture were: J. J. Albert (West Virginia), P. A. Arneson (New York), D. Chandler (Washington, J. N. Cummins (New York), R. S. Dickey (New York), W. M. Dowler (Maryland, J. D. Gilpatrick (New York), J. E. Hunter (New York), E. J. Klos (Michigan), J. W. Lorbeer (New York, R. C. Lamb (New York), W. R. Landis (New Jersey), J. L. McIntyre (Connecticut), W. J. Moller (California), K. G. Parker (New York), R. C. Pearson (New York), D. H. Petersen (Pennsylvania), J. L. Preczewski (New York), T. M. Sjulín (Illinois), P. W. Steiner (Missouri), D. H. VanEtten (New York), J. Van Geluwe (North Carolina), A. D. Woods (New York)

THIRD WORKSHOP ON FIRE BLIGHT RESEARCH

Statement of Receipts and ExpendituresRECEIPTS - Goods

Proceedings Printing, Binding and Postage

USDA - ARS

RECEIPTS - Cash

Donations from Agri-chemical firms

\$1,000.00

[Merck Chemical (Research), Merck
Chemical (Development), I.C.I.,
US, Stauffer Chemical Corp.,
Ciba-Geigy Corp., E.I. duPont de
Nemours, Inc., Pfizer, Inc.]

Personal Donation (Prof. K. G. Parker)

25.00

Full Registrations (40 @ \$10)

400.00

Partial Registrations (8 @ \$2.50)

20.00

Bar-B-Q Admissions (7 @ \$5)

35.00

Total \$1,480.00

EXPENSES

Bus Charter

\$ 152.75

Meeting Refreshments

87.96

Reception

112.04

Bar-B-Q

248.10

Meeting Expenses (microphones, tapes,
guest meals, name tags, etc.)

39.73

Travel Assistance

760.64

Program Printing

19.83

Misc. Gaphics and Printing

20.64

Total \$1,441.69

BALANCE

+ \$ 38.31

The Apple and Pear Disease Workers
acknowledge with thanks the contributions
of the following companies toward costs of
the Workshop:

Agway, Inc.

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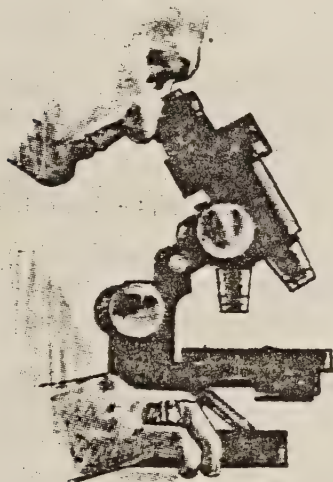
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 S. V. Beer, Cornell University, Ithaca
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REGISTRATION

To cover costs of the workshop, all attendees must register. Full workshop including bus trip and barbecue: \$10.00. Monday and/or Wednesday only: \$2.50

All meals taken in Ramada Inn must be billed to room or paid in cash after meal.



Sunday, September 19, 1976

5:00 p.m. Welcoming Reception and Registration (Room 147)
 6:30 Dinner (independently)
 8:00 Welcoming Reception and Registration (Room 147)

Monday, September 20, 1976

7:30 a.m. Breakfast (together)
 Opening Remarks: S. V. Beer (Ithaca)
 Welcome by W. K. Kennedy, Dean,
 College of Agriculture and Life
 Sciences, Cornell University

8:30 Registration

8:40 EPIDEMIOLOGY

Epiphytic and internal *Erwinia amylovora*, monitoring, bacterial strands, weather, etc.

S. V. THOMSON (Berkeley), *Chairman*;
 S. V. Beer, E. Billing (East Mall-
 ing), G. Bonn (Harrow)

10:00 Coffee break

10:15 Cankers, dissemination of pathogen,
 bacteriophages, *Erwinia herbicola*,
 etc.

E. J. KLOS (East Lansing), *Chairman*;
 D. F. Ritchie (East Lansing), S. V.
 Beer, A. K. Chatterjee (Davis)

12:00 Noon Lunch (Buffet, together)

1:30 p.m. PHYSIOLOGY

Host ultrastructure, amylovorin,
 bacterial agglutination, etc.

R. N. GOODMAN (Columbia), *Chair-
 man*; T. M. Sjulín (Urbana), S. V. Beer,
 L. N. Gibbons (Guelph), A. K. Chatter-
 jee

3:15 Coffee break

3:30 Biochemistry, tree nutrition, induced
 host resistance, etc.

J. KUC (Lexington), *Chairman*; H. L.
 Keil (Beltsville)

6:00 Dinner (Group outing to Stone House Inn)

8:00 **CONTROL MEASURES**

Chemical control, insects, streptomycin resistance, biological control, etc.

H. L. KEIL, *Chairman*; S. V. Thomson, W. J. Moller (Davis), G. Bonn, M. Abdel-Rahman (Syracuse), S. H. Davidson (Wilmington), S. V. Beer



Tuesday, September 21, 1976

7:30 a.m. Breakfast (together)

8:30 Field Trip to Research and Commercial Orchards in Wayne County, NY

12:00 Noon Lunch

1:30 p.m. Tour of Fire Blight Field Experiments at New York State Agricultural Experiment Station, Geneva

3:00 Coffee break in Plant Pathology Department, Geneva

3:15 Tour of Research Facilities and Greenhouse Fire Blight Experiments

5:30 Barbecue Supper at Geneva

8:00 **"CURRENT STATUS AND EPIDEMIOLOGY OF FIRE BLIGHT IN EUROPE"**

W. Zeller (Germany)
C. A. R. Meijneke (Netherlands),
E. Billing (England)

Wednesday, September 22, 1976

7:00 a.m. Breakfast (together)

8:00 **CAUSAL ORGANISM**

Virulence, pathogenicity, genetics, selective media, strains, etc.

M. N. SCHROTH (Berkeley), *Chairman*; A. K. Chatterjee, L. N. Gibbons, D. F. Ritchie, S. M. Ries (Urbana)

9:30 Coffee break

9:45 **RESISTANCE**

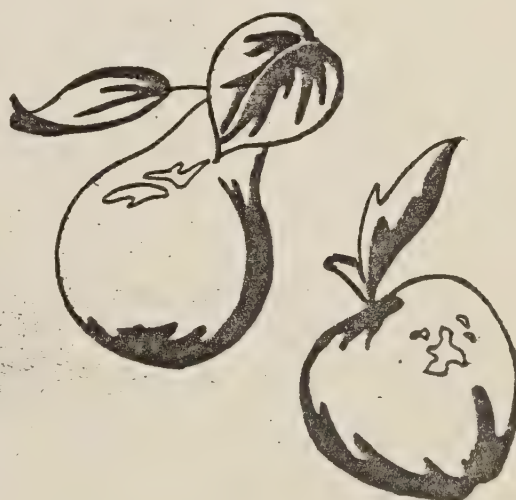
Sources, inheritance, seedling screening, breeding—apples, pears, rootstocks.

H. S. ALDWINCKLE (Geneva), *Chairman*; S. V. Beer, E. Billing, J. N. Cummins (Geneva), G. Bonn, T. van der Zwet (Beltsville)

11:30 Lunch

1:30 p.m. Field Trip to Test Plots of Agway, Inc., Fabius, NY

M. Abdel-Rahman



EPIDEMIOLOGY

EPIDEMIOLOGY OF FIRE BLIGHT BLOSSOM INFECTION

Steven V. Beer
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The blossom blight phase of fire blight reduces the current season's crop, may be the starting point for severe tree damage, and provides inoculum for later infection stages. In recent years, blossom blight control has received more attention than other phases because it is more easily controlled by chemical means. In general, the bloom period is limited and thus the protectant or weak eradicant control materials, now available, can be applied during a definite time segment. Vegetative shoot infection is more difficult to control because shoots are susceptible for a longer time during the growing season.

Because of the importance of blossom infection, studies were undertaken to determine the influence of several factors on its development. Two types of studies were carried out. Under precisely-controlled conditions using blossoms on potted trees held in controlled-environment chambers, individual blossoms were inoculated with 10 μ l of suspensions containing known numbers of viable Erwinia amylovora cells [Ann. Appl. Biol. 81:159 (1976)]. Variables included in these experiments were inoculum dose, incubation temperature, nutrient status and cultivar. In another series of experiments, apple blossoms on trees in a research orchard were inoculated by spraying the blossom clusters with suspensions containing known concentrations of Erwinia amylovora. The effects of inoculum dose, time of inoculation and cultivar on the amount and rate of infection development were evaluated.

The orchard studies formed the basis for the development of techniques used to assess various materials for the control of blossom blight.

Most of the experiments were done with inoculum that had been prepared in advance. A single colony isolate of Erwinia amylovora was grown in broth shake culture. Log phase cells were harvested by centrifugation, resuspended in skim milk and frozen at -20 C. (Techniques of inoculum preparation and tree inoculation are described in detail in an article that will shortly appear in Fungicide and Nematicide Techniques, a new book scheduled to be published by APS.)

For the precisely controlled studies, potted dormant trees were stored at 2 ± 2 C. Trees were grown in a greenhouse until shortly before bloom when they were placed in controlled-environment chambers. All blossoms not at the proper stage of development were removed and the remaining were hand-pollinated. Usually, about 25 spurs containing 3-6 similar blossoms each were inoculated per treatment. Ten microliter amounts of E. amylovora suspensions were placed on the receptacle surface (without wounding) with an Ependorff pipette. Individual blossoms were examined at 8 hr intervals for the development of symptoms. Symptomatic blossoms had ooze on the

exterior surface of the receptacle or peduncle and water soaking and necrosis typical of fire blight infection [Proc. Am. Phytopathol. Soc. 2: 95 (1975)].

The time required for development of symptoms was negatively correlated with incubation temperature and inoculum dose. When the inoculum dose exceeded ca. 10^4 cells per blossom, the final amount of infection was independent of incubation temperature. However, the final amount of infection was positively correlated with incubation temperature with an inoculum dose of ca. 10^2 cells per blossom. Differences in the amount of infection that developed when pear cultivars were inoculated with identical inoculum doses were more apparent at low doses. The results of these experiments raised questions concerning E. amylovora population dynamics in inoculated blossoms.

Changes in numbers of E. amylovora cells in inoculated blossoms were followed by harvesting individual blossoms at intervals and washing or grinding them in sterile buffer. The macerates or washings were dilution-plated on Crosse and Goodman's medium [Phytopathology 63:1425 (1973)] to determine viable cells per blossom. During the first 12 hrs after inoculation of pear blossoms with ca. 10^4 cells per blossom, at 21 C, a rapid decline in number of E. amylovora cells recovered was noted. More cells were recovered from blossom macerates than washings suggesting movement of the bacteria into the internal blossom tissues. By 24 hrs, cells recovered had increased to approximately the same level as had been applied at inoculation. After 24 hrs, the number of cells recovered increased logarithmically to a maximum of ca. 10^8 cells per blossom at 6 days when symptoms were well developed. The proportion of blossoms with E. amylovora populations in excess of 5×10^6 , 4 days after inoculation when no symptoms were apparent, was approximately equal to the percent of symptomatic blossoms 5 days after inoculation. The changes in E. amylovora numbers at 21 C were studied also in 0.05 M potassium phosphate buffer, nutrient broth and Kado's 523 broth. In the buffer, E. amylovora numbers declined steadily until none were detectible after 48 hrs. A slight lag period was apparent in the nutrient broth before logarithmic increase occurred. No lag period was observed when E. amylovora was transferred to the enriched Kado 523 medium. In contrast, E. amylovora numbers in inoculated pear blossoms declined sharply for the first 12 hrs and then increased. These data suggest that blossoms may inhibit or kill E. amylovora cells initially and the ultimate amount of infection may be related to the number of blossoms in which the bacteria survive the lag phase of development.

In field studies on apple similar results regarding the rate and amount of infection development as a function of inoculum dose were noted. Rate of infection development and ultimate amount of infection were positively correlated with inoculum concentration. In addition, more infection developed more rapidly in blossoms of the Twenty-Ounce cultivar than in blossoms of the Idared cultivar.

It is hoped that these data may form the basis for the construction of a mathematical model of blossom blight which may be useful in predicting the need for control measures and disease severity.

Fireblight predictionEve Billing^{1/}

A system has been developed, based on temperature and rainfall records, which seems to have predictive value in south-east England and is under test elsewhere.

An assessment of the potential doublings (PD) of the pathogen per day is obtained from a table based on in vitro growth rates at different temperatures (Billing, 1974). This combined with a rain score (R), derived from field experience, is used to determine the length of an incubation period (I) using an equation derived from previous studies (Billing, 1976). The incubation period is deemed to end when

$$I \leq \frac{\{R. (\sum PD + 6)\}}{36}$$

The degree of precision of this system remains to be tested but there was a good correlation ($r = 0.907$, $P < .001$) between the number of completed incubation periods per season and the number of infected trees reported over the period 1959 to 1968 (omitting 1963).

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2007
OCCURRENCE OF FIRE BLIGHT STRIKES DURING THE GROWING SEASONW. GORDON BONN¹/

Fire blight is a major limiting factor in the pear industry in Ontario. The disease has repeatedly reached the epiphytotic level in many orchards throughout the province and has forced growers to limit pear acreage and the potential yields. The common control method for fire blight has been a reduction in fertilizer applied to the trees and the subsequent decrease in fruit production.

A major survey in the early 60's as well as several others in the 70's for pear fire blight indicated that most losses occurred as a result of twig blight. Blossom blight was also reported, however only one was ever confirmed as fire blight. Thus, it would appear that losses in pears were due to twig blight and its extension into older wood.

The current fire blight study was undertaken to determine the incidence of twig blight in a commercial orchard throughout the growing season. Individual fire blight strikes were counted on a weekly basis from blossom through to mid September.

Blossom blight was not observed during the past three seasons and this was consistent with previous observations on the lack of blossom blight in this area. Twig blight, on the other hand, was first observed at the beginning of June and continued through to the last observation date (Fig. 1). During June the number of new fire blight infections was low but increased dramatically in July and then decreased in August. Since the period of greatest damage occurs during a relatively short period in mid-summer, appropriately timed applications of spray materials may effectively reduce losses due to twig blight.

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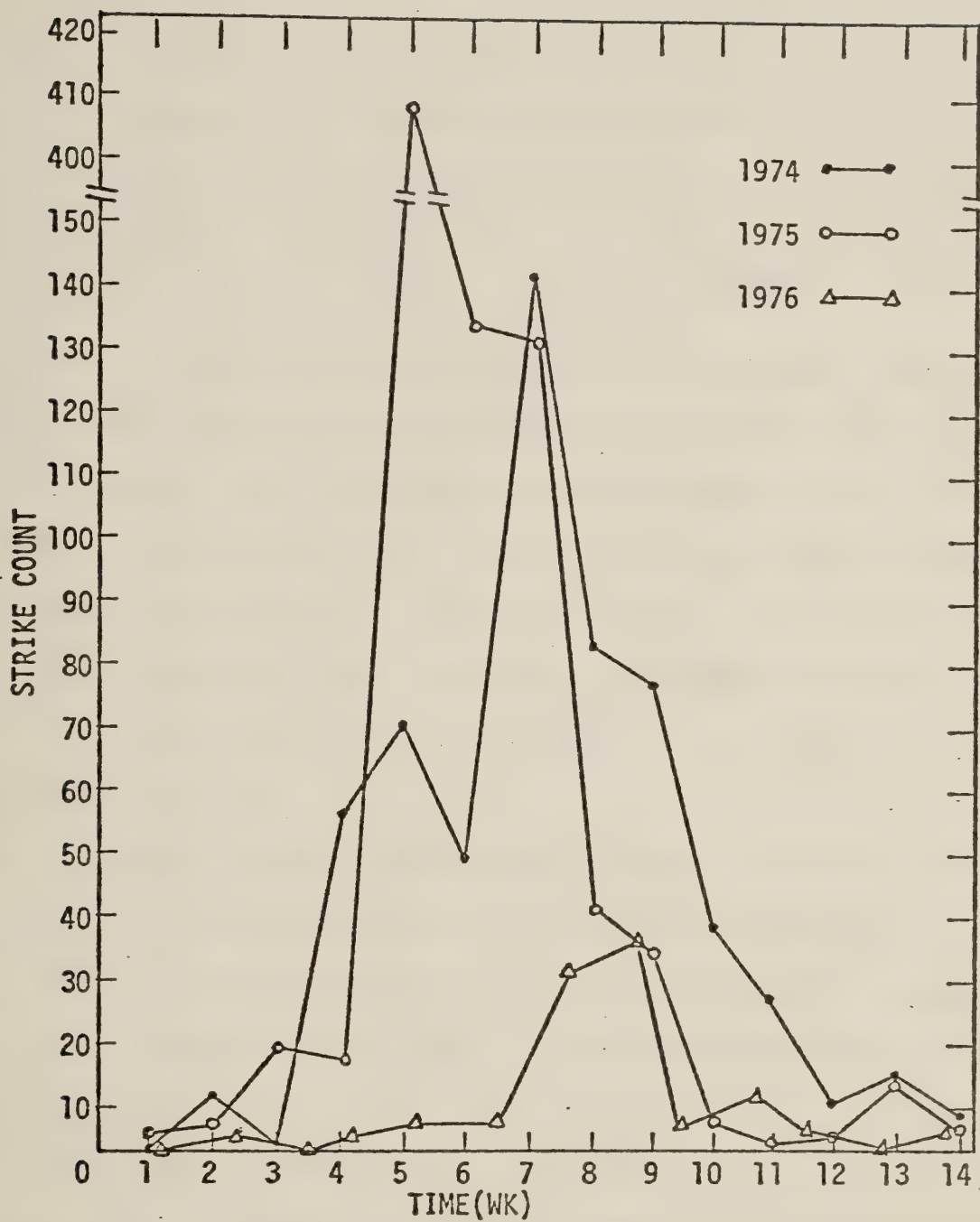


FIG. 1. OCCURRENCE OF FIRE BLIGHT STRIKES
THROUGHOUT THE GROWING SEASON

EPIDEMIOLOGY (Part I)

Discussion of S. V. Thomson's Presentation

A = Aldwinkle	G = Goodman	M = Moller	Bl = Blake
Be = Beer	Ke = Keil	S = Schroth	C = Cummins
Bi = Billing	Kl = Klos	T = Thomson	D = Davidson
Bo = Bonn	Ku = Kuch	vZ = Van der Zwett	

- G: Those that have considered the osmotic concentration of nectar, and I don't know whether they've worked with the stigmatic fluids, but I know that nectar seems to be a feature that either permitted or precluded infection, that is, if the osmotic concentration of the fluid--of the nectar--was too high, you had no bacterial multiplication. So I wonder whether or not the stimulation was rain or the addition of rain is actually a dilutant of the osmotic concentration of, say, stigmatic fluids, and nectar, to give you then an atmosphere in which bacteria can grow.
- S: In examining the work on sugar concentration in media, I could not find any real evident support that nectar concentrations influence infection. It appears as if it was purely hypothetical. We know that Erwinia amylovora can multiply in high concentrations of sugar. Your own work using the sucrose medium indicates this also.
- G: Well, you know, that occurred to me too. You know, I think that the osmotic... well, if you take a look also at Shaw's work, osmotic concentration is in fact a very strong feature in the rate of growth. I mean, as you increase osmotic concentration you can reduce bacterial growth significantly. My own medium, as you know, provides only restricted growth and I think the medium that you work with also has that sort of restriction, whether it's placed on osmotic concentration, I don't know. But I do think that it's worth considering or reconsidering. I'm not saying that that's the fact.

Bi: I don't think it's a straight osmotic effect, but I'm sure those water relationships are terribly important, from our experience. And I wonder whether these populations you find in blossoms are in the middle of their incubation period. (I'll be talking about incubation period at the time of infection, at the time of symptom expression.) And later in the season, but I think not only in the season, if whether it's dry, I think the things will stick. And so, yes, water in some form must be.... Now I've done some experiments some time ago with growth of the bacteria at different osmotic concentrations of sucrose, and I was surprised at what happened. It increased the lag, but once the lag was over, within the limits of my experiments, the rate, at quite high concentration dropped to They're rather incomplete experiments, but the osmotic thing is not simple and straightforward, and I don't think water relationships are straightforward. But water, I swear, is the key in our work.

T: Certainly the bacteria are capable of multiplying on the stigmatic surface, which, by the way, does not contain carbohydrates. There may be other compounds which influence the osmotic pressure, but there are no simple sugars such as glucose or fructose.

Bi: It's different at different times of day.

G: There's an experiment that comes to mind--I don't think it's been done--at least I haven't read about it, but if one was to put seedlings or plants under stress, under moisture stress, where you could actually calculate the amount of moisture in a shoot at a given time, and then inoculate under those condition. Would you be able to suppress infection or create a longer lag by putting the plant under greater moisture stress? What I'm considering is that intercellular space is the area where the bacteria must be growing. If you decrease the amount of available moisture, you also decrease the amount of moisture in the intercellular space. And there too, you may be affecting, pardon the expression, "osmotic concentration."

D: Bob, I haven't done that experiment exactly, but I have worked with flacid seedlings which are perhaps 3-4 weeks old. The total number of plants expressing or manifesting disease will be greater if the plants have their normal turgor and the total number manifesting disease will be less if the plants are slightly to excessively flacid. These are not blossoms, you understand, these are just 3-4 week-old seedlings.

A: How do you inoculate them?

D: I just atomize the inoculum at about 10⁵ over the plants.

-: No wounds?

D: No wounds. It's a very pathogenic strain that Harry Keil has and you don't have to wound to get disease to take. But it does go down if the plants are flacid and the line will stay flat or slightly raised if they are turgid.

A: What sort of seedlings are they?

D: Romes.

Discussion of S. V. Beer's Presentation:

Bo: Steve, in that last slide you showed us, were those blossoms protected from outside bacteria coming in after you had inoculated them?

Be: No.

Bo: I was just wondering about when you inoculated with a low concentration of bacteria with an incubation period of twenty days, whether that wasn't caused by bacteria that were present in the orchards?

Be: It could have been, but I don't think it was, because under our conditions bloom doesn't last very long, and we know that as the blossom ages it becomes less and less susceptible, so I think we would have to have inoculation occurring within a day or two after our official inoculation in order to have that.

G: I'm not sure I understand that question raised by Gordon. Which slide are you referring to?

Bo: The last slide he showed.

G: The one where there was a drop at the end of one day?

Bo: No, I was looking at the low concentration levels in which there were no symptoms until approximately twenty days later.

Ke: I don't think that's an unusual response, it was just normal field infection, oftentimes we will see no blight on blossoms in the field, but some three weeks later we will see some blight that we can associate back with the blossom period.

Kl: Why is that Harry?

Ke: Well, I really don't know. I think there's a big lag because of the temperature or environment, or something like that, Ed. That's the only thing that I would suspect.

Be: I'd like to mention just one other thing. We did a comparison because we were seeing this lag in the number of bacteria recovered after inoculation. We wanted to see what that really means. We put a given number of bacteria in a phosphate buffer and then followed the number that we could recover and it went down kind of like that.

G: What was the concentration at the outset?

Be: It would be a thousand per 10 lambda, because it was the same concentration that we placed in the pear blossom. So, in phosphate buffer, you got something like this at 21 C. If we put it in nutrient broth, we get a bit of a lag and then it comes up. If we put it in Kado 523, which is a medium, kind of like nutrient broth, with lots of sucrose in it, there's no lag. Now, if you put it in pear blossoms, we find there is a sharp decline to 12 hours, and then it takes 24 hours to get back to where we started, and then we get a log increase.

Bi: Lelliott has found just this same thing.

Be: It struck me as sort of a possible killing effect by the blossoms. I just present this, and wonder what other people would think of it.

G: In addition to that I think that these are two separate effects: the bacteria which are put in buffer is decay that one sees in a medium such as water without any protection of the bacterial cell. If the numbers of bacteria are low. If you use 10^6 cells per ml, you don't pick up that decay. But if you put in from a thousand to 100 cell per milliliter in distilled water, in half an hour you can't find live bacteria. They just decay so quickly. But I believe that the line that you drew, Steve, for the blossom feature, that's something else again. I think that's more than just natural decay in an unprotected medium.

Ke: Well I think Covey and Coyer had similar data to this which was from field blossoms actually where they inoculated trees in the field and it showed exactly what you say.

Discussion of Eve Billing's Presentation

T: Prior to infection, can you predict when disease will occur?

Bi: No, only on the past, depending on the time of year you're talking about - if there have been a lot of things like this at the beginning of the season then I would just use my common sense in effect and say there's a high potential. If it falls again before a previous one, the incubation depends entirely on the weather reports at the place you got your fire blight. I can show you one diagram where I got that situation. Mostly I am taking East Malling weather data and looking at fire blight somewhere in Kent and rainfall can be so variable that if you score one weather station you can only predict rather broadly what's going on.

M: In California, sometimes we see infection that apparently is unrelated to rain.

Bi: It may be your heavy dews - We don't get such heavy dews. It's wetness that matters, and persistent wetness - heavy dews. Do you have foggy periods during flowering periods?

M: Not too much, that may happen in some years but most years we can't even relate it to that.

G: How about root pressure. Have you measured things like that? Root pressure or water movement from leaf surfaces.

M: No.

Bi: You see, early in the season I think the water relationships are much less important - I needn't use my equation; all I need to think about is temperature. But I think we need the rain to start it off, a heavy dew will initiate things, and if you don't get too hot and dry in between.

S: Our program of monitoring is designed to reduce the number of pesticide applications. There is no way we can predict infection. Infection is a very complicated matter and it depends on the susceptibility of the flowers, various environmental conditions, and factors influencing the previous years.

Bi: If you have a dry autumn, I think there may be less chance, you haven't got these indeterminant cankers factors perhaps, but it's not as simple as that, I can't simply say that; we had a wet autumn, therefore we're going to have trouble next year. I can say we have had a wet autumn, therefore there is a very good chance for infections next year.

S: I think it is important to remember that geographical localities play an important role in how the disease operates. Our trees are not very susceptible. We can bring in flowers, inoculate them and have a cloudy drop of bacteria in the receptacle without infection occurring. Thus it is really the unusual flower that becomes infected in California.

Bi: What population do they reach?

S: You can have populations of 10^7 - 10^9 without infection - at other times you can inoculate rat tail flowers and have a high incidence of infection.

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Bi: Well, I am only making claims about primary blossom infection in apple and pear and general fire blight activity in all hosts through the season. Usually we're dealing with something like Hawthorne blossom at the end of May, Pear usually blossoms all through the summer and Cotoneaster. I think my equation works best from mid May on.

G: In 1966 I happened to be in Kent in a pear orchard where they had rows of Laxton superbs and Bartletts side by side. And Laxton Superbs were showing tremendous amounts of fire blight in its rat tail bloom, whereas Bartlett (you call it Williams) was completely free of fire blight.

Be: No rat tail blossoms or no rat tail infection?

G: No rat tail blossoms.

vZ: Well, you are talking about two degrees of susceptibility.

Bi: This is characteristic and so I would always - surely you must ____ with you people you get much more severe storms early in the season.

vZ: The same two varieties in the same conditions, one is much more susceptible than the other plus one had rat tail blooms.

G: I think that what we're seeing is something that is seen in California as well. If their susceptibility is extended because they have vulnerable tissue for a longer period of time.

Bi: And we all know that whatever system we devise we've always got to take into account all the other things: what syndrome, did you have a storm, or just a shower of rain, and things like that.

Be: I'd like to ask Milt Schroth how he could explain the observation of 10^7 , 10^8 , 10^9 E. anylovora per blossom, in a cloudy suspension without any infection.

S: I cannot! People have been working on the physiology of parasitism for a long time and in general can not explain why plants become susceptible or resistant. Susceptibility is somewhat inherent in the plant, yet also depends on environmental conditions.

Bi: It's a very complicated situation.

- S: It's extremely complicated. We also cannot explain why infections run sometimes, and other times they don't. We've worked on glucosides as has Joe Kuch but we cannot attribute susceptibility simply to this presence or absence.
- G: The term I couldn't think of before, Bill, was the hydathodes. Do you see any guttation from leaves... I just wonder if root pressure and guttation wouldn't be the key achieved for California conditions.
- M: Yes.
- S: Many times you can find large guttation drops in flowers in the morning which later disappear. This would seem to provide a good opportunity for fire blight bacteria to invade the flowers, particularly if the droplet moves back into the flower. However, it still depends on whether or not the flower is susceptible.
- G: What do you mean by susceptible?
- S: Susceptible to infection. Even during one of our heavy epidemic periods in 1971, where every single flower was infested, we seldom have much more than a 100 flowers infectious per tree, which is in contrast to your experience. Why those 100 flowers per tree are more susceptible than the other ones can't be readily explained.
- Bi: Could it be that they had a lower dose of bacteria?
- S: An examination of the flowers reveal a similar amount of infestation.
- Be: Where are the bacteria located besides on the surface of the flower?
- S: I can't tell you, we didn't look in those times. I would assume they were on the stigmatic surface.
- : Were the bacteria applied?
- T: No
- Be: Under natural conditions where are the bacteria located, in infested blossoms versus infected blossoms.
- T: In infested blossoms, the bacteria are only found on the stigmatic surface. In infected blossoms the bacteria are on all flower parts.
- : Is it possible there's injury involved in those 100 blossoms over the whole tree that was infected?

- S: I really doubt it. It's like Pseudomonas syringae. It is present all over our trees and causes epidemics just during certain years.
- A: How do you explain Steve Beer getting such a high proportion of his flowers infected with inoculum of 10^7 per flower? It seems like nearly all of the flowers that were inoculated developed infection.
- Ke: It's an entirely different situation. In California they were found on the stigmatic surface by Steve Beer is inoculating the nectaries.
- S: If the inoculum is applied in the receptacle with an eppendorf pipette you do not obtain much infection. If the inoculum is sprayed, you get more infection. More parts of the flower apparently are inoculated and bacteria invade the hydathodes and nectaries - etc.
- T: We obtain only 10-20% infection if we apply bacteria in the receptacle.
- G: Spraying the inoculum increases infection?
- T: That's right.
- G: So therefore, if we just project a little bit, if you get the bacteria into an area where they are in fact protected and prevented from drying, let's assume into the nectary per se, where they can begin to multiply.
- T: The droplet is applied directly to the nectaries however when you spray, more portions of the flower are covered.
- G: Not necessarily. The nectary is much like a stomatal opening you have to get it into the substomatal chamber or the nectarial chamber per se and it takes pressure to do that.
- T: If you place a droplet on a flower it will be drawn back in within just a few minutes.

Bi: Then you haven't got the same water relationships do you?

T: We get similar results in the greenhouse, in the lab, and in the field.

-: Isn't there a greater likelihood that when you're spraying your inoculum that you may be getting a lot of microinjuries on the cells?

T: I doubt it. We use an aerosol mist which is not a coarse spray.

S: We can also spray E. amylovora on trees and not obtain infection. I've also done it and had an epidemic.

Ke: This is nature. This is a synchronization of many many factors, we may talk about the environment, we may talk about the plant, we may talk about the flower and there are many many things we may talk about. But the reason they get 100 blossoms and only 100 blossoms in there most severe year, is that there is a proper synchronization of the factors that allow infection to take place. It only took place in those 100 flowers out of many thousands. That's the way I would see it, now if we're ever able to put all these factors together and synchronize them - it's really inconceivable that we can do that.

Discussion of G. Bonn's Presentation:

Question?

Bo: No. 1 here would correspond to the end of the first week in June.

M: Then what are your average temperatures during bloom?

Bo: A maximum might be 65, a minimum 45, but very very erratic, our bloom period lasts approximately 6-10 days.

G: Eve Billing, isn't that something like the temperatures you get in apples in Kent, these kind of temperatures. So you don't normally have blossom blight in Kent?

Bi: We don't normally have blossom blight in Kent even when temperatures are high enough we don't always have it. But according to my system there aren't enough potential doublings accumulated. Incidentally I didn't say my potential doublings is not unlike the degree - Day system.

-: What if you have a storm during bloom?

Bi: Yes, that might start something, but unless you've got something happening before you've got to have your inoculum and this is the sort of thing that I'd very much like to discuss with people as to what's going on in early spring. This is still a difficult area.

Be: Gordon Bonn, you indicated that you started to pick up epiphytic populations in May. How late into the summer do you detect them?

Bo: Until about the middle of August.

-: As you're counting your strikes are you distinguishing between those twigs which are extending from vegetative buds and those which are rising from the blossom? If you have a fruit bud, it will send up two twigs. Are you distinguishing in your strikes between the shoots that are coming from vegetative buds and those which have arisen from blossom buds, or from mixed buds, in which the blossom has fallen off?

Bo: No, I'm not.

-: What I'm really getting at here is that you may have had an influx of bacteria during blossom time which had no visible effect upon the blossom and then later on is affecting one of the two shoots that has arisen from this bud.

Bo: Why would we see the symptoms developing from the tip backwards in that case?

Be: Is this actually what you're seeing?

Bo: From the tips backwards...The flugging, darkening and finally some oozing.

G: That's normal sequence, I mean if you inoculate a shoot on a median leaf and incubate for 72 or 84 hours, the first symptoms that you see are at the apex or the pedicel of the leaf that you inoculated. You see, no symptoms or no damage along the entire length of the shoot and its the apex that shows symptoms, so I think that the point raised by Jim is a relevant one. It's quite possible you have a latent infection that isn't expressed because of the low temperatures.

Bi: I was looking for this with Gordon Bonn because I've ~~met this~~ and I know from greenhouse experiments where I have infections first expressed in the tip and just from what I saw it looked like mostly straight tip infection. I think this has to be taken into account that your infected blossoms may rub off. The first sign of infection is in the tip, later on.

B1: Did you mention the fact that you couldn't find blight in the orchard at blossom time. You didn't find bacteria on the flowers by washing them.

Bo: No we didn't find it that period at that time.

B1: Well this eliminates the flower phase of it then, you couldn't find it during flower time.

Bo: During the flowering time or for the next two weeks following petal fall?

-: That is a pollinated fruit, not a pollinated ruit, in the subsequent fruit.

Bo: We see some infection, but that would be on the rat tail, rat tail blossom and we don't really have too much of that in our area.

T: I have two questions: What medium are you using and do you see insect activity such as leaf hoppers or pear psylla at the time of shoot tip infections?

Bo: Cross + Goodman's. We do have psylla. This year we had a bad case of psylla, and very little fire blight. I think the insects may be involved but they don't play as large a part as rainfall or some of the very damaging storms that come through our area.

-: I assume you are removing strikes so that you don't have to count them again. But when you do remove a noted strike do you feel that you're affecting your inoculum source and might see less strikes later on.

Bo: This is why we picked an area within the middle of an orchard that has a history of fire blight and in most years, the level of fire blight would be high enough to provide a good source of inoculum for the small plots that we have within.

B1: Do you keep track of wind movements, in addition to rain at the time.

Bo: We have all the data but we haven't gone over it yet.

Ku: I must join the ranks of the puzzled. I don't mind being puzzled because when I visit my M.D. I also realize that he's puzzled. But, I don't understand why it is, however, that the things that Dr. Thomson _____ that apparently he couldn't detect the bacteria on the unopened parts, the infection of the bacteria and the multiplication occurred in the flower in the stigma, but apparently this was a non-specific site and other bacteria could also multiply there. I assume that the reason the Erwinia is found there and multiplies is because it's sticky. It is an environment which they can divide in. It puzzles me why, in your case, you don't find bacteria on the blossoms. I assume the source of the bacteria are cankers that are already there, on the tree. Why would there be such a difference if it's non-specific, sticking to this stigma, multiplying, why would there be such a difference in finding and not finding the organism.

Bo: This is why we suspect that cankers are active but the rainfall, but we don't have high enough temperatures that build up the potential doublings to such a point that infection is going to show up in a short span of time. Bacteria may be present there. We have not been able to detect them, but if they go ahead and infect it may take three to four weeks for symptoms to develop, whereas in the middle of summer, it may take only two or three days, because we do have a lot of fluctuating temperatures during pre-bloom and postbloom period and bloom period in ontario. Temperature influences the rate of multiplication of the bacteria after infection has taken place. And also, the development of inoculum in the old cankers.

T: When we have temperatures similar to that in California, we don't find bacteria in the flowers either. In fact, early in the season we did not find bacteria in the flowers because the temperatures were too cool. It takes temperatures exceeding a maximum of 65 F. We start finding bacteria in flowers when the maximums exceed 80 F.

G: It also says something about the plating efficiency of the media that we use. It may be that we're really not able to recover the one or two cells that are present and because of the decay factor that Steve's data shows ... and others have noted.

Be: The decay factor is different at different temperatures. At 4 degrees C., the temperature that we use to collect blossom samples, canker swabbings and so on, Erwinia amylovora is most stable in phosphate buffer versus distilled water, versus dilute media, very dilute media, versus saline. But at 21 C. it's not stable. The graphs that I drew before were at 21 C., which was the incubation temperature of the blossoms. We thought that was comparable. I might mention that in our monitoring, we have been able to detect Erwinia amylovora on the surface of unopened blossoms. This was detected only on blossoms that were in the immediate vicinity of cankers, cankers that either prior to, or simultaneously also yielded Erwinia amylovora.

G: Are you suggesting internal migration from the canker?

Be: No, however that could happen, but I assume a more simple mode of transfer by rain from the canker surface to the flower.

A: You said the surface of unopened blossoms. Do you mean the outside of the blossom, not the stigmatic surfaces inside?

Be: No, this is taking blossom buds and washing them.

2011
Isolation and Partial Characterization of Erwinia amylovora

Bacteriophages from Aerial Tissues of Apple Trees //

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Bacteria like many other organisms are subject to infection by viruses. Viruses of bacteria, termed bacteriophages, phages, or simply viruses, were first reported in 1915 by Twort (12). Subsequent research has shown that most if not all bacteria including plant pathogenic bacteria have viruses which can infect them (4,7, 13). Being obligate parasites phages can usually be detected wherever their host is found. Some phage-bacteria interactions have been studied in great detail, e.g. Escherichia coli and its phages. Among the phytopathogenic bacteria, phages of Xanthomonads and Pseudomonads have been primarily studied though phages for all genera are known (5,7,13). In the case of Erwinia amylovora, the causal organism of fire blight, much work has been done with the pathogen and the disease (11). Some effort has been made to study the interaction of E. amylovora with other microorganisms but in very few cases have the phages of E. amylovora been studied (3,6). In 1973, Erskine reported the results of his study dealing with the interaction of E. amylovora and its phage (6). However, not until 1976 had E. amylovora

phages been reported to occur on aerial tissues (9). This report describes the isolation and partial characterization of E. amylovora phages from aerial tissues of apple trees during 1975 and 1976.

In June of 1975, phages of E. amylovora were isolated without enrichment from symptomless and diseased tissues of apple trees at Michigan State University (MSU). Phage was also detected on similar tissues in two commercial orchards in southwestern Michigan. The methods used for collecting and washing the tissue were the same as used for monitoring E. amylovora populations (11), utilizing a differential medium for detecting E. amylovora (10). The methods used in detecting and handling the phage were essentially as outlined by Adams (1). E. amylovora strain #110 was used as host unless stated otherwise.

Table 1 summarizes the types of tissues sampled and the approximate populations of E. amylovora and phages obtained from these tissues. The data show that phage could be isolated from most aerial tissues as long as E. amylovora was present.

The time of the year during which phage could be detected on foliar tissues is shown in Figure 1A. Figure 1B shows the populations of E. amylovora and its phages during the months of phage detection. The 1975 results were obtained from monitoring the orchard at MSU while 1976 results were obtained from monitoring 10 growers' orchards in southwestern Michigan.

During both years phage were detected in mid-June, three to four weeks after the initial outbreaks of fire blight. High populations of E. amylovora were present prior to the detection of phage with the highest populations of E. amylovora detected during the first half of June. This coincided with the period during which the most rapid spread and greatest number of new strikes occurred. As this period reached its maximum and leveled off phages were detected with the phage population reaching its maximum from mid-June to mid-July. This corresponds with a period of static fire blight activity; e.g. very few new strikes occurring. It should also be noted that this is the period of terminal growth cessation. The E. amylovora and phage populations declined from a peak in early July to a level of no detection in late August to early September. From September 1975 to April 1976 more than 200 cankers were sampled from throughout the state while 10 apple and 10 pear cankers at MSU were periodically sampled during the same time period. At no time were phages detected even in the cases where enrichment was used while in 40% of the cankers E. amylovora was detected. Failure to detect phage does not necessarily mean that they may not be present (7). It appears that phage are not detected until an "explosive" growth of E. amylovora has occurred, if this is the case it may be indicative of the phage possibly existing as a prophage. Also, it may be necessary for the host bacteria to be in a rapidly growing state for infection

and replication to occur. This condition would not be likely to occur in Michigan from September through April.

Some factors, other than the presence of a host, which may affect the ability of phage to survive on foliar surfaces are desiccation and ultraviolet light (UV). These two factors were tested in the laboratory using Jonathan apple leaf discs 1.3 cm. in diameter. On each disc was placed 10^8 plaque forming units (PFU) of phage isolate PEa1(h) suspended in 0.02 M potassium phosphate buffer. One set was placed on a screen over water in a closed, clear plastic box such that the relative humidity remained near 100%. A second set was placed on paper towels in a pan with relative humidity ranging from 15-25%. The data (Fig. 2A) indicate that the phage is very sensitive to drying since a 100,000 fold decrease occurred within 24 hours, while at the high humidity the phage concentration remained stable for the first three weeks then dropped sharply. The sharp decline coincided with the occurrence of leaf tissue degradation and decay. Phage inactivation may be due to enzymatic activity.

To study the effect of UV light, the same procedure was used. Approximately 10^5 PFU were placed on each leaf disc then the discs were exposed to varying periods of UV; 254 nm wavelength at a distance of 8.0 cm. There was a 1000 fold decrease in phage after 60 seconds of exposure (Fig. 2B).

The extreme sensitivity to desiccation may explain the decline of phage during July and August. It is during this time that the blighted terminals and leaves become very dry.

A list of 16 phage isolates and some of their characteristics is shown in Table 2. Over the last two years four major plaque types produced by the phages have been observed. The most frequent isolated type produced a plaque 4-5 mm in diameter with an extending halo (Fig. 4). The second type which was isolated in association with PEa1(h) and PEa2 produced a clear plaque 1-3 mm in diameter without a halo but having an irregular margin (Fig. 4). It has not been possible to completely separate the halo type from the non-halo type but very easy to separate the non-halo type from the halo type. There are also intermediates of halo production. The reason for the halo could be due to lysogeny but is probably due to a polysaccharide depolymerase (2). It is also interesting to note that PEa1(h), PEa1(nh), and PEa12 are temperature sensitive and will not grow at 30 C but grow well at 27 C. This is not due to their failure to infect since they can tolerate temperatures of 90 C and still infect E. amylovora (Table 2).

The third plaque type, exemplified by PEa7 and PEa15, is a small, 1-2 mm plaque with an irregular margin and an extending halo. The phage producing this plaque type are different from the PEa1(h) types because they are able to grow on E. amylovora "mutants" resistant to PEa1(h).

The fourth and most interesting phage type is produced by isolates PEa9, 10, and 11. One interesting characteristic is their extreme sensitivity to chloroform and ethyl ether. The reason for this is not presently known. The major problem in working with this phage type is that we have not been able to devise a purification system to separate the phage from host bacteria.

The one-step growth curve for isolates PEa1(h), PEa2, and PEa5 is shown in Figure 3. The phage have a latent period of approximately 35 minutes, a rise period of 30 minutes and a burst size of 45-50 PFU/productive cell.

The host range of the phages is shown in Table 3. Phage solutions of 10^6 - 10^7 PFU/ml were spotted on top agar seeded with an 18 hour nutrient broth culture of the test bacterium and incubated at 27 C for 24 hours. Only E. amylovora strains were lysed even when tested over a temperature range from 10 C to 30 C. Clearer areas of lysis were formed when incubated at 10-20 C than when incubated at 24, 27, and 30 C.

In a living, changing system no entity exists as an isolated individual. Microorganisms on foliar tissues of plants are not exempt from this interaction (8). Within these microbial ecosystems, composed of biological and non-biological components, there is interaction with each organism competing with other organisms for survival. If these microbial ecosystems exist on a larger

biological organism (host) any change that affects the microbial ecology often ultimately affects the host many times resulting in what is termed a diseased condition for the host. This is the situation not only for animals but also for plants. E. amylovora exists in a complex ecosystem affected not only by physical factors such as weather but also by many biological factors. First, it is associated with a host that passes through many complex physiological changes each year. On this host it must also compete with many other microorganisms such as bacteria, fungi, and viruses. A better understanding not only of the biology of E. amylovora but also of the organisms with which it interacts could make it possible to better understand the disease which it causes, fire blight.

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Table 1. Aerial tissues of apple trees associated with Erwinia amylovora and E. amylovora bacteriophages. Sampling was done during June and July of 1975 and 1976.

<u>Tissue Sampled</u>	<u>Erwinia amylovora</u>	<u>Bacteriophage</u>
Diseased terminals and leaves of Jonathan	25 of 25 terminals 1.0 X 10 ⁵ CFU/g	25 of 25 terminals 4.0 X 10 ⁴ PFU/g
Diseased Jonathan leaves	5 of 5 samples with 5 leaves/sample ~10 ⁶ CFU/g	5 of 5 samples with 5 leaves/sample 5.0 X 10 ⁴ PFU/g
Diseased Jonathan stems without leaves	5 of 5 samples 5 stems/sample ~ 10 ⁶ CFU/g	5 of 5 samples 5 stems/sample 3 X 10 ⁵ PFU/g
Symptomless Jonathan terminals and leaves	8 of 25 terminals 5 X 10 ⁴ CFU/g	4 of 25 terminals 2 X 10 ³ PFU/g
Symptomless Jonathan leaves	5 of 5 samples with 50 leaves/sample ~10 ³ CFU/sample	0 of 5 samples with 50 leaves/sample 0
Newly formed Jonathan twig cankers	14 of 14 cankers 10 ⁶ - 10 ⁷ CFU/g	12 of 14 cankers ~10 ⁵ PFU/g
Diseased McIntosh leaves and terminals	8 of 10 terminals ~10 ⁵ CFU/g	9 of 10 terminals ~ 10 ⁵ PFU/g
Diseased Red Delicious terminals and leaves	9 of 10 terminals ~ 10 ⁵ CFU/g	10 of 10 terminals ~ 10 ⁵ PFU/g
Diseased Golden Delicious terminals and leaves	10 of 10 terminals ~ 10 ⁵ CFU/g	5 of 10 terminals ~ 10 ⁴ PFU/g
Diseased Jonathan apples	6 of 6 apples 4 X 10 ⁸ CFU/g	4 of 6 apples 7 X 10 ³ PFU/g

Colony Forming Units - CFU
 Plaque Forming Units - PFU

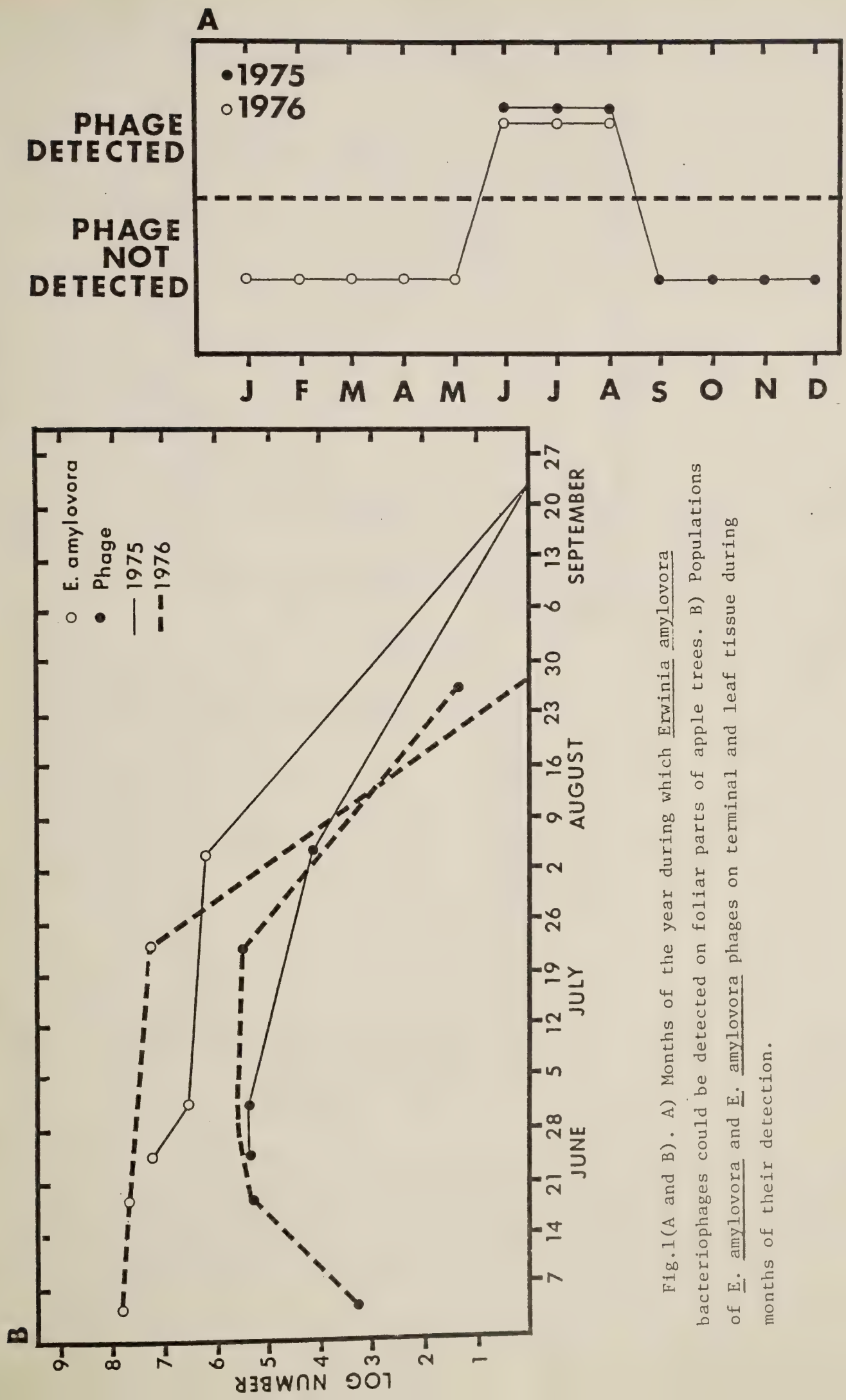


Fig. 1(A and B). A) Months of the year during which *Erwinia amylovora* bacteriophages could be detected on foliar parts of apple trees. B) Populations of *E. amylovora* and *E. amylovora* phages on terminal and leaf tissue during months of their detection.

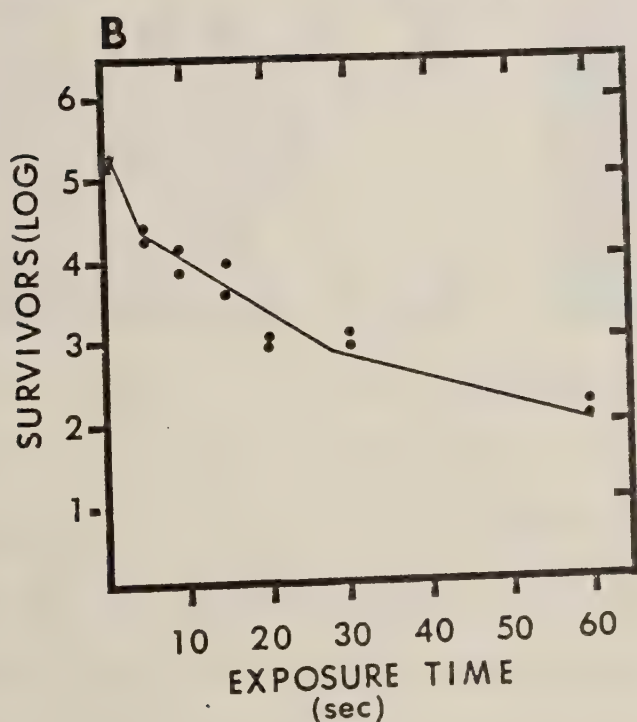
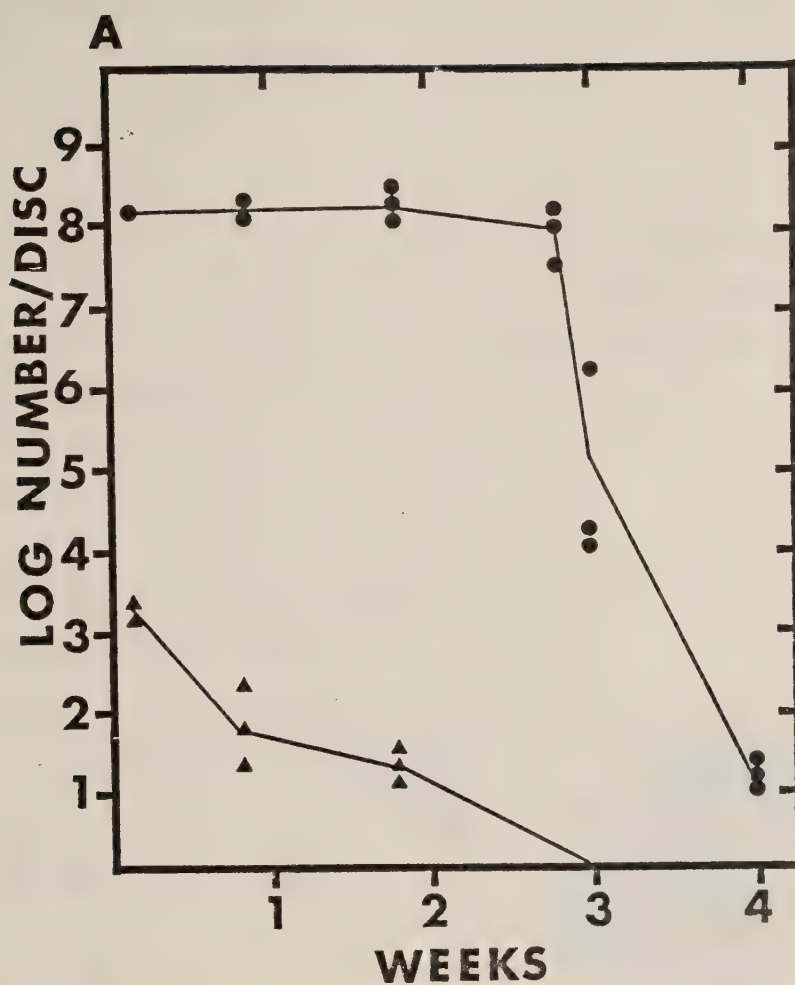


Fig.2.(A and B). A.) Ability of phage PEal(h) to survive on Jonathan apple leaf discs at 90-100% relative humidity (●—●) and at 10-25% relative humidity (▲—▲). B.) The ability of PEal(h) to infect *E. amylovora* following exposure to ultraviolet light at 254 nm at a distance of 8.0 cm.

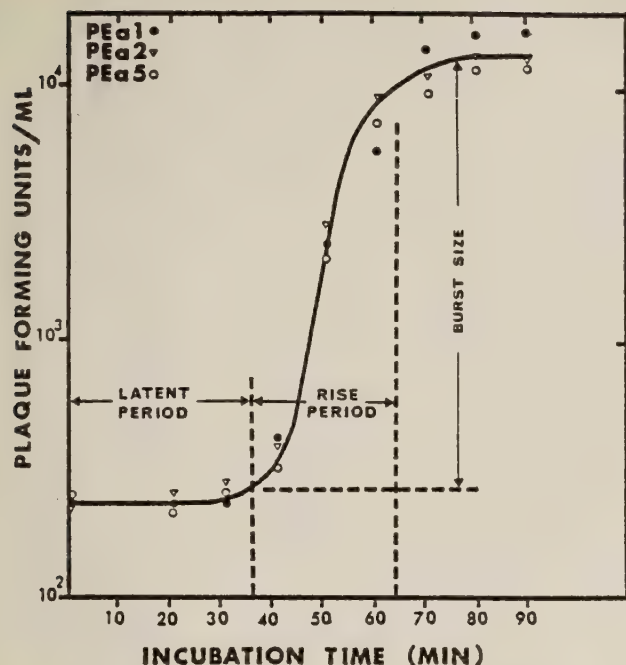


Fig. 3. One-step growth curve of phages PEa1, PEa2, and PEa5 with Erwinia amylovora #110 as host.

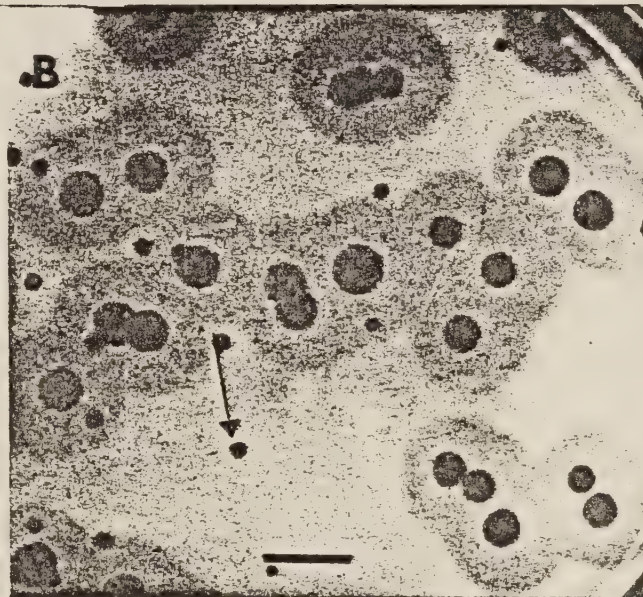
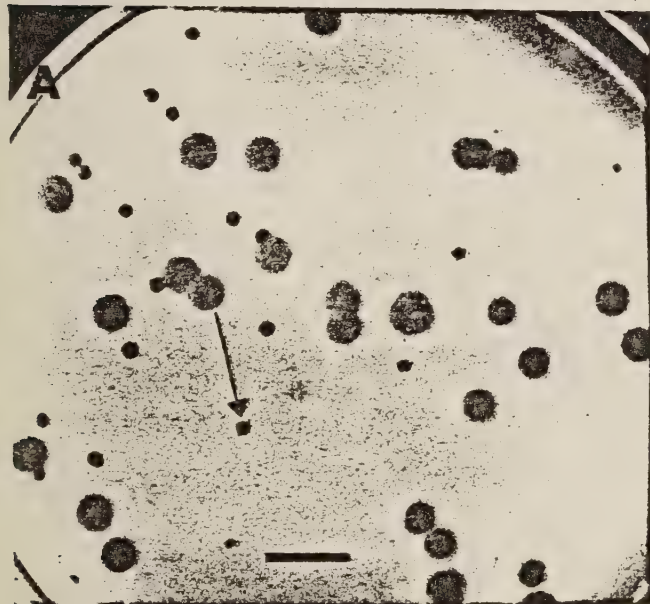


Fig. 4-(A and B). A) Plaque morphology of phage PEa1 (halo) and PEa1 (non-halo), arrow, after 18 hours incubation at 27 C with Erwinia amylovora #110 on 2.5 ml of top-agar consisting of 0.7% nutrient agar, 0.5% glucose, and 0.5% yeast extract. B) Plaque morphology of phage PEa1 after 48 hours under the same conditions as in A. Note the extending, translucent halo of PEa1 (halo) while the plaque morphology of PEa1 (non-halo) has remained the same as at 18 hours (arrow). The scale bar equals 1.0 cm.

Table 2. Sixteen phage isolates and some of their characteristics.

Phage Isolate	Location of Isolation	Source		Plaque Morphology		Chloroform sensitive
		Blighted Jonathan terminals & leaves	Other	4-5 mm plaque with extending halo	Other	
PEa1(h)	Paw Paw	+		+		-
PEa1(nh)	Paw Paw	+			1-3 mm without halo	-
PEa2	MSU		Soil	+		-
PEa3	MSU		Diseased Jonathan fruit	+		-
PEa4	MSU		Newly formed Jonathan canker	+		-
PEa5	MSU		Symptomless Jonathan terminal	+		-
PEa6	MSU		Diseased pear terminal	+		-
PEa8	Lawrence	+		+		-
PEa12	Watervliet	+		+		-
PEa13	Paw Paw	+		+		-
PEa16	MSU(Hort)	+		+		-
PEa7	Berrien Springs		Diseased crabapple terminal		1-2 mm with halo	-
PEa15	MSU(Hort)	+			1-2 mm with halo	-
PEa9	MSU(Hort)	+			2-4 mm without halo	+
PEa10	Paw Paw	+			2-4 mm without halo	+
PEa11	Berrien Springs		Diseased crabapple terminal		2-4 mm without halo	+

Phage Isolate	Ability to replicate at 30C or greater	Ability to lyse <u>E. amylovora</u> isolates resistant to PEal(h)	Temperature at which no survivors were detected following 10 minutes of heating
PEal(h)	-	-	90 - 100 C
PEal(nh)	-	-	n.d. ¹⁾
PEa2	+	-	90 - 100 C
PEa3	+	-	90 - 100 C
PEa4	+	-	90 - 100 C
PEa5	+	-	90 - 100 C
PEa6	+	-	90 - 100 C
PEa8	+	-	n.d.
PEal2	-	-	n.d.
PEal3	+	-	n.d.
PEal6	+	-	n.d.
PEa7	+	+	55 - 65 C
PEal5	+	+	55 - 65 C
PEa9	n.d.	n.d.	n.d.
PEal0	n.d.	n.d.	45 - 50 C
PEal1	n.d.	n.d.	n.d.

1) not determined - n.d.

Table 3. Host range of Erwinia amylovora bacteriophages.

Bacterial isolate	Bacteriophage Isolate												
	<u>PEal(h)</u>	<u>PEal(nh)</u>	<u>PEa2</u>	<u>PEa3</u>	<u>PEa4</u>	<u>PEa5</u>	<u>PEa6</u>	<u>PEa7</u>	<u>PEa8</u>	<u>PEa12</u>	<u>PEa13</u>	<u>PEa15</u>	<u>PEa16</u>
<u>Erwinia amylovora</u>													
104 Cal.	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy
116 Spinks Corners	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy
137 Hartford	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy	hazy
105 I11	+	+	+	+	+	+	+	hazy	+	+	+	hazy	+
110 MSU	+	+	+	+	+	+	+	hazy	+	+	+	hazy	+
112 Traverse City	+	+	+	+	+	+	+	hazy	+	+	+	hazy	+
113 G.Rapids	+	+	+	+	+	+	+	hazy	+	+	+	hazy	+
115 Paw Paw	+	+	+	+	+	+	+	hazy	+	+	+	hazy	+
118 Cal	+	+	+	+	+	+	+	hazy	+	+	+	hazy	+
119 MSU	+	+	+	+	+	+	+	-	+	+	+	hazy	+
120 N. Car.	+	+	+	+	+	+	+	hazy	+	+	+	hazy	+
121 N. Car.	+	+	+	+	+	+	+	hazy	+	+	+	hazy	+
124 Paw Paw	+	+	+	+	+	+	+	hazy	+	+	+	hazy	+
134 Paw Paw	+	+	+	+	+	+	+	hazy	+	+	+	hazy	+
<u>Erwinia herbicola</u>													
YH-1 MSU(Hort)	-	-	-	-	-	-	-	-	-	-	-	-	-
#7 Berrien Springs-	-	-	-	-	-	-	-	-	-	-	-	-	-
#8 Hartford	-	-	-	-	-	-	-	-	-	-	-	-	-
#10 Paw Paw	-	-	-	-	-	-	-	-	-	-	-	-	-

The following bacteria were also tested but were not lysed: Agrobacterium tumefaciens UC3416 and UC78, Corynebacterium fascians, C. flaccumfaciens, Enterobacter aerogenes, Erwinia atroseptica SR 8, E. carotovora SR 165, Escherichia coli, Pseudomonas aeruginosa, P. fluorescens, P. lachrymans, P. syringae, Serratia sp., Xanthomonas pruni PF-2.

FIRE BLIGHT CANKERS - CHARACTERISTICS OF INOCULUM PRODUCERS,
DETECTION AND PREVENTION OF ACTIVITY

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In areas where fire blight is endemic, the pathogen overwinters primarily at the margins of cankers formed the previous year. Bacteria which are dormant during the winter commence multiplication at the margins during the spring or early summer. If multiplication is sufficient, great numbers of bacteria are exuded through the bark and accumulate on the surface in ooze. The bacteria in ooze may be disseminated to new infection courts by rain, insects or other vectors. Because of their importance in inoculum production, we have attempted to learn more about cankers that successfully overwinter the pathogen and the dynamics of inoculum production.

The purpose of these studies was to determine the characteristics of those cankers that do successfully overwinter the pathogen and release inoculum, the factors influencing inoculum release and possible ways of inhibiting inoculum release.

A technique was developed to monitor pathogen release from cankers under orchard and greenhouse conditions. Canker margin surfaces and adjacent healthy tissues were swabbed with sterile cotton-tipped hospital applicators, previously dipped in sterile 0.05 M potassium phosphate buffer, pH 6.5. The swabs were washed in buffer and the washings were plated on the *Erwinia* selective media developed by Crosse and Goodman [Phytopathology 63:1425 (1973)], and Miller and Schroth [Phytopathology 62:1175 (1972)]. Bacteria which grew on the media that were similar in colony morphology to authentic *E. amylovora* were reisolated and tested for pathogenicity on immature apple or pear fruit [Phytopathology 66:317 (1976)].

Studies of cankers in commercial orchards have indicated that those with smooth (indeterminate margins) located on larger branches have a much higher probability of releasing the pathogen than those with rough (determinate) margins. We have found that *E. amylovora* can be recovered from canker margin surfaces that do not have macroscopically visible evidence of ooze, perhaps explaining the frustration of several earlier workers when they failed to discover oozing cankers in orchards newly affected by fire blight. *E. amylovora* was recovered from canker margin surfaces during several discrete time intervals suggesting that environmental factors influence pathogen activity in cankers.

In a test designed to determine if moisture, external to the canker surface, affected the probability of cankers producing *Erwinia amylovora*, matched pairs of cankers were identified based on margin character, size of branch bearing the margin, location in the trees, and age of

tissue infected. One member of each pair was covered by a feminine sanitary napking, previously saturated with sterile water. Erwinia amylovora was recovered from a significantly higher proportion of the cankers that had been covered with the moist pads than those that had not been covered. Thus, it appears that external moisture affects the probability of recovering Erwinia amylovora from canker surfaces. In contrast, in another experiment, cankers were covered with plastic "umbrellas", constructed of wire mesh and polyethylene sheeting. The pathogen was recovered from a somewhat smaller number of the covered cankers than those without umbrellas.

To determine if the time of infection influences the type of canker formed, and subsequent production of Erwinia amylovora, 2-year-old Idared trees were inoculated with E. amylovora in July and in September in the nursery row. After leaf fall (in November) all trees were dug and graded for margin characteristics. Eighty-nine percent of the trees that had been inoculated in July had formed determinate margins. In contrast, 72% of the trees inoculated in September had formed indeterminate margins. The times of inoculation used may be related to natural infection; infections initiated early generally result from blossom infection, whereas vegetative infection that develops following hail storms is comparable to late inoculation.

Trees with cankers were subsequently potted in a mixture of peat and vermiculite and incubated in controlled environment chambers at 17, 21 or 28 C. Cankered trees with indeterminate margins produced E. amylovora (as determined by weekly swabbing) in significantly greater proportion than cankered trees with determinate margins. Trees incubated at 21 C produced the pathogen in higher proportions than those incubated at either higher or lower temperatures. Although statistical analysis of the data, indicated that incubation temperature had a significant effect, the numbers of trees involved were small [Proc. Am. Phytopathol. Soc. 3: in press (1976)].

Bordeaux mixture (8-8-100) plus 1 or 2% superior 60'oil applied at the green-tip to 1/4" (6 mm) green growth stage has been recommended for fire blight control in New York and in several midwestern states for several years. The mode of action of this combination in reducing fire blight infection is not known. Cankers on Bartlett pear trees were selected for similarity in margin characteristics, branch size, and location. Half of the selected cankers were treated by air-blast sprayers with Bordeaux mixture (8-8-100) plus 1% oil. The other half were not treated. Each canker margin surface was swabbed twice weekly starting at 1/4"-green for 11 weeks. Until petal fall, Erwinia amylovora was recovered from 11 of the 20 unsprayed cankers. During the same period, none of the Bordeaux mixture + oil-treated cankers yielded the pathogen. The effect of the pesticide appeared to wear off with time as in the 6 week interval after petal fall, eight of the treated cankers yielded the pathogen, and an additional five untreated cankers yielded the pathogen. These results suggest that Bordeaux mixture+oil applied early during the growing season reduces fire blight infection later, by inhibiting inoculum production by overwintering cankers.

The removal of fire blight cankers from orchard trees has been a recommended control measure for many years. Complete canker removal is accomplished rarely because it is often difficult to distinguish cankers from healthy tissues. As part of a research program designed to determine the feasibility of remotely detecting fire blight, the infrared light reflectance from fire blight cankers was determined with a spectroradiometer. In contrast with comparable healthy bark, cankered bark reflected very little infrared light. Based on these physical differences, infrared sensing techniques were developed to permit more ready distinction of cankers than can be accomplished with the human eye. Between 700 and 900 nanometers little infrared light is reflected from cankered tissues. Thus, pictures made with infrared-sensitive false-color film, show cankered tissue as grey-blue, whereas comparable healthy bark appears magenta. This difference in infrared reflectance of cankered tissue may also be sensed with electronic infrared viewers, such as the Find-R-Scope, manufactured by F. J. W. Industries, Mount Prospect, Illinois. For proper operation, both the electronic sensor and photographic sensors must be used with filters to remove light of less than 700 nanometers [Proc. Am. Phytopathol. Soc. 2: 34 (1975)].

Continued study of the epidemiology and control of E. amylovora production by fire blight cankers may lead eventually to predictive systems based on analysis of physical rather than biological characteristics. Additional effort is needed to find effective materials to prevent cankers from producing E. amylovora thus eliminating inoculum production by overwintering cankers.

Genetic interaction between Erwinia herbicola and other enterobacteria
including Erwinia species

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15 September 1976

The bacteria known as Erwinia herbicola are ubiquitous in nature. While found predominantly as epiphytes (on plants), these bacteria are also detected in such diverse habitats as soil, hospitals, humans, and other animals. Under certain conditions, these organisms are involved in fatal infections in compromised human patients; they are certainly a nuisance in nosocomial infections. In relation to plant diseases, these (seemingly nonpathogenic) bacteria appear to confer a protective effect against infection by bacterial pathogens. Notable in this context is the protective effect of Erwinia herbicola on apples against infection by Erwinia amylovora (Goodman, 1965, 1967; Riggall and Klos, 1972; McIntyre, Kuc, and Williams; 1973; Wrather, Kuc, and Williams, 1973). Other biochemical properties of these bacteria which are of interest to plant and medical bacteriologists are now being unravelled. It has been reported that some strains of E. herbicola can fix atmospheric nitrogen (Neilson and Sparell, 1976; Potrikus and Breznak, 1976). We have observed that some of these bacteria can metabolize a variety of organic compounds not metabolized by other Erwinias or other enterobacteria. This metabolic diversity perhaps reflects a nutritional adaptation for survival and multiplication in special habitats in nature. In order to fully understand, and perhaps to fully exploit, the traits of these bacteria, a full spectrum of genetic and biochemical studies is needed. In our laboratory in Davis, we are at present studying the following aspects of those subjects:

- a. Plasmid inheritance, which might explain the ability of these bacteria to cause infections in humans.
- b. Transfer of genetic material, including the genes controlling pigmentation, from Erwinia herbicola to Erwinia amylovora and other enterobacteria, and the effect of such transfer on virulence of the latter organisms.
- c. Lysogeny and bacteriocinogeny. Genetic and molecular studies are being performed to determine whether any of these traits are plasmid-borne in these bacteria and whether or not they are transmissible. Lysogenic phages are being tested for transducing ability. In addition, we have now constructed P1 clr100Cm-lysogens of E. herbicola strains, and we are testing the induced phage for generalized transduction. We are also attempting to develop a P1-sensitive line from the P1 clr100Cm-lysogen.

- d. Metabolic diversity. A systematic and comprehensive study is in progress [Table 1]. We are in the process of testing the ability of these bacteria to fix atmospheric nitrogen. Our studies on the utilization of L-histidine as the source of carbon and/or nitrogen has revealed that these bacteria resemble Klebsiella aerogenes in this respect. A detailed study to determine the regulation of the hut operon in E. herbicola is in progress. The degradation of arbutin and phloridzin by these bacteria, and the possible role of the degradation products in altering the course of the fire-blight disease has been documented (Chatterjee and Gibbins, 1969; Chatterjee et al., 1969). Since these bacteria also confer a protective effect on other host-pathogen systems (Hsieh and Buddenhagen, 1974), it might be possible that the degradation of other compounds by these metabolically versatile bacteria might be involved in other such specific host-pathogen systems.
- e. We have found that, in a mixed culture, strain EH105 (Y74) of Erwinia herbicola causes a loss in viability of the cells of Shigella dysenteriae strain 2880-S₁ [Table 2]. This loss in viability of S. dysenteriae cells does not result from a decrease in pH of the growth medium, nor from bacteriocins or bacteriophages that the E. herbicola strain might produce. The mechanism of this unusual inhibitory effect, and its possible relevance to E. amylovora systems, are presently being explored by us.
- f. Genetic and molecular properties of the Erwinia plasmid, E-lac⁺. This transmissible plasmid, detected by us (Chatterjee and Starr, 1973) in human clinical strains of E. herbicola (see below), has a wide host range, and is remarkably stable in a variety of phytopathogenic enterobacteria. These properties of this plasmid make it suitable for the development of conjugal gene transfer systems in other groups of Erwinia spp., notably the members of the "carotovora" group, in which we have now developed a conjugal gene transfer system (Chatterjee and Starr, in preparation). This system is not yet as efficient as the gene transfer by our Hfr strains of Erwinia amylovora, but we expect that the use of the derepressed mutants of the sex factor E would enable us to develop a more efficient conjugal gene transfer system in this group, as well as in other groups of plant bacteria.

For the purposes of this Workshop, we will confine our discussion to (a) plasmid inheritance in E. herbicola, and (b) the properties of the Erwinia sex plasmid, E-lac⁺. The results will demonstrate that genetic exchange between E. herbicola and E. amylovora does indeed occur under laboratory conditions and, hence, possibly occurs in nature as well.

The transfer from other enterobacteria to strains of E. herbicola of plasmids such as F'lac⁺, R100 drd-56, and SR1 occurs readily [Table 3]. The frequency of such transfer is low in relation to the transfer of these plasmids to E. coli K12 strain. However, all these plasmids, except for SR1, are remarkably stable in these hosts. The sex factor activity in E. herbicola strains harboring the transmissible plasmids, as in E. coli, is derepressed--as judged by sensitivity to the male-specific phage in the case of the F' factor or R100 drd-56, and by the frequency of the transfer of these plasmids to Erwinia spp. and to other enterobacteria [Table 4]. It is noteworthy that the plasmid-bearing strains of E. herbicola exhibit a higher level (about 5-fold) of tetracycline (Tet) resistance compared to the donor E. coli and Shigella flexneri cultures. It is possible that the replication of the plasmid in E. herbicola hosts is relaxed--as opposed to a stringent control of replication of the plasmid in E. coli. The relaxed replication would produce multiple copies of the plasmid, and the higher level of resistance perhaps results from a gene dosage effect.

Recently, we have found that the plasmid RPI is readily transferred from E. coli to E. herbicola, and that E. herbicola strains harboring RPI are capable of transferring the plasmid to Erwinia spp. and to other enterobacteria. These results demonstrate that plasmids are readily transferred from enterobacteria to E. herbicola strains, and that the "herbicola" strains retain the plasmid under non-selective conditions, and can donate the plasmids to a variety of enterobacteria including other Erwinia spp.

At this juncture in our consideration of the case, we argue that (if such transfer of plasmids between E. herbicola and other enteric bacteria is indeed occurring in nature) we should be able to detect the presence of plasmids in natural isolates of E. herbicola. To that end, we undertook a systematic search for plasmids in human clinical strains of E. herbicola. A collection of such strains was obtained from Mount Sinai Hospital in New York (from E. Bottone), and we initially examined the isolates for their

biochemical properties. Although these isolates resembled plant strains of E. herbicola in cultural and biochemical properties, some strains differed among each other and from plant strains with respect to a few characters. The characters of interest to us were (a) drug resistance and (b) the ability to utilize lactose as the sole source of carbon and energy. The lac genes are known to be carried on a variety of plasmids in enterobacteria. In light of our findings on $F'lac^+$ transfer between E. coli and E. herbicola strains, it was deemed worthwhile to test the lac⁺ strains for the presence of plasmids. Out of nine strains tested, we detected the ability to transfer lac⁺ in two strains of E. herbicola (EH122 and EH133); such transfer was effected to other strains of E. herbicola (from animals and plants) and to Shigella dysenteriae. The frequency of this transfer was low with E. herbicola strain EH122 and slightly higher with EH133. We subsequently established that this transfer was mediated by conjugation and not by a bacteriophage, although the donor strains (EH122 and EH133) are lysogenic and could be induced by mitomycin C treatment, and the phage particles plaqued on lawns of related E. herbicola strains. The host range of the E-lac plasmid is rather broad [Table 5]. E-lac is readily transferred to Erwinia spp., to E. coli, to Salmonella typhimurium, and to Shigella dysenteriae. The plasmid is very stable: we have not detected the spontaneous loss of the plasmid thus far from any of the hosts that we have tested. It is noteworthy that this E-lac plasmid, in contrast to $F'lac^+$, is remarkably stable in Erwinia chrysanthemi; $F'lac^+$ is lost from cells of this species at a very high frequency (85 to 90% of the total population becomes Lac⁻ when grown under non-selective conditions), whereas we have not detected any Lac⁻ clones from E. chrysanthemi strains harboring E-lac⁺ under identical experimental conditions. This particular trait, along with the extended host range of this plasmid, makes it more suitable for genetic work on Erwinia spp.

We have tested the lac⁺ exconjugants for their donor ability. The results [Table 6] reveal that lac is transferred from all the exconjugants tested; the frequency of transfer varies, depending on the donor strain employed. That the sex factor E is linked to lac⁺ genes, and that therefore the sex factor and lac⁺ genes are cointegrates as opposed to aggregates, is suggested by the results of Plkc transduction of E-lac⁺ in E. coli host system. At present, we are seeking physical evidence for this state by

isolating the plasmid DNA, transforming E. coli cells to the Lac⁺ state, and then testing the Lac⁺ clones for their donor ability.

The results presented above clearly indicate that plasmid transfer to E. herbicola strains from enterobacteria occurs readily. The detection of the plasmid E-lac⁺ in the human clinical strains of E. herbicola suggests that such interaction among these bacteria occurs in nature as well. Thus far our results do not reveal a significant change in the virulence of plasmid-bearing strains either of Erwinia amylovora or of members of the "carotovora" group. However, this aspect is currently under systematic examination in our laboratory.

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TABLE 1.

COMPOUNDS UTILIZED BY ERWINIA SPR AS THE SOLE SOURCE
OF CARBON AND ENERGY

<u>COMPOUND</u>	<u>"HERBICOLA"</u>	<u>"AMYLOVORA"</u>	<u>"CAROTOVORA"</u>
D-ARABINOSE	+	-	-
D-GALACTURONIC ACID	+	-	+
D-ALANINE	+	-	-
L-ORNITHINE	+	-	-
γ-AMINOBUTYRATE	+	-	-
L-HISTIDINE	+	-	-
L-PROLINE	+	-	-
PUTRESCINE	+	-	-
TRIGONELLINE	+	-	-
ARBUTIN	+	-	-
PHLORIDZIN	+	-	-

TABLE 2.

EFFECT OF ERWINIA HERBICOLA STRAIN Y74 AND ITS LAC^+
EXCONJUGANT Y74 R3 ON THE VIABILITY AT 30C OF SHIGELLA
DYSENTERIAE STRAIN 2880 S1

TREATMENT	VIABLE COUNT (NO./ML)	
	0 TIME	3 HR
E. HERBICOLA Y74	1.3×10^9	5.5×10^9
E. HERBICOLA Y74 R3	1.2×10^9	5.8×10^9
S. DYSENTERIAE 2880 S1	7.2×10^8	1.7×10^9
E. HERBICOLA Y74 x S. DYSEN- TERIAE 2880 S1		
Y74	1.5×10^9	7.0×10^9
2880 S1	7.0×10^8	5.0×10^4
E. HERBICOLA Y74 R3 x S. DYSENTERIAE 2880 S1		
Y74 R3	1.1×10^9	4.7×10^9
2880 S1	7.5×10^8	3.2×10^4

TABLE 3.

TRANSFER OF $\text{F}'\text{LAC}^+$ AND R-FACTORS FROM
ENTEROBACTERIA TO ERWINIA HERBICOLA

SPECIES	PLASMID	FREQUENCY OF TRANSFER OF DONOR CELL TO	
		EH103	EH105
ESCHERICHIA COLI K12	$\text{F}'\text{LAC}^+$	3.6×10^{-6}	7.7×10^{-6}
E. COLI B/R	R100DRD-56	1.2×10^{-4}	1.0×10^{-4}
SHIGELLA FLEXNERI	SR1 (Tet^R Chl^R STR^R)	1.2×10^{-5}	1.0×10^{-6}

TABLE 4.

TRANSFER OF PLASMIDS FROM ERWINIA HERBICOLA TO ERWINIA SPP.
AND OTHER ENTEROBACTERIA

RECIPIENT SPECIES	PLASMIDS TRANSFERRED		
	F' <u>LAC</u> ⁺	R100 <u>DRD</u> 56	SR1
ERWINIA AMYLOVORA	+	+	-
ERWINIA CHRYSANTHEMI	+	+	-
ERWINIA CYTOLYTICA	+	+	-
ERWINIA HERBICOLA	+	+	-
ESCHERICHIA COLI	+	+	-
SALMONELLA TYPHIMURIUM	+	+	-
SHIGELLA DYSENTERIAE	+	+	-

TABLE 5.

TRANSFER OF LAC FROM HUMAN CLINICAL ERWINIA STRAIN EH133
TO OTHER ENTEROBACTERIA

RECIPIENT	FREQUENCY OF <u>LAC</u> TRANSFER (PER DONOR CELL)
ERWINIA AMYLOVORA (EA178-S ₁)	4.7×10^{-7}
ERWINIA CHRYSANTHEMI (EC16-S ₁)	2.2×10^{-7}
E. HERBICOLA (EH103-S ₁)	1.4×10^{-6}
E. HERBICOLA (EH105-S ₁)	7.6×10^{-8}
E. HERBICOLA (EH106-S ₁)	3.4×10^{-7}
E. HERBICOLA (EH126-S ₁)	3.4×10^{-8}
ESCHERICHIA COLI (2492)	1.0×10^{-7}
SALMONELLA TYPHIMURIUM (3335-S ₁)	2.0×10^{-8}
SHIGELLA DYSENTERIAE (2872-S ₁)	1.8×10^{-6}

TABLE 6.

DONOR ABILITY OF THE LAC^+ EXCONJUGANTS HARBORING $E-LAC^+$ (ORIGINALLY FROM HUMAN CLINICAL ERWINIA HERBICOLA STRAIN EH133) TO ESCHERICHIA COLI F^-LAC^+ STRAIN 2492- N_1

DONOR CULTURE :	FREQUENCY OF LAC TRANSFER (PER DONOR CELL)
ERWINIA AMYLOVORA (EA178- S_1 - L_1)	6.0×10^{-5}
E. HERBICOLA (EH103- S_1 - L_1)	2.0×10^{-5}
E. HERBICOLA (EH105- S_1 - L_1)	1.5×10^{-6}
E. HERBICOLA (EH106- S_1 - L_1)	1.0×10^{-4}
ESCHERICHIA COLI (2492- L_1)	4.3×10^{-4}
PARACOLOBACTRUM ARIZONAE (2236- S_1 - L_1)	2.0×10^{-4}
SALMONELLA TYPHIMURIUM (3335- S_1 - L_1)	1.0×10^{-4}

Pertinent Statements Made During Discussion on Epidemiology (Part II)
E. J. Klos, Chairman

This session is a continuation of the first section and discussions that followed on epidemiology with emphasis on cankers, dissemination of the pathogen, bacteriophages and Erwinia herbicola.

CANKERS

Generally it is agreed that cankers are the major primary source of inoculum in the spring. Cankers are broadly divided in two types determinate and indeterminate. The former having definite demarcation between diseased and healthy tissue and the latter showing a color differentiation without any definite demarcation. Isolation of the bacteria from the indeterminate cankers generally shows a higher percentage of success. Some researchers suggest size of cankers influenced the survival of bacteria, however others have shown evidence disagreeing with this statement.

Presence of ooze on cankers was thought to be the chief indicator of primary inoculum. Beer and Scroth indicated that bacteria could be isolated from canker surfaces without evidence of ooze. Beer stated that cankers kept moistened artificially yielded the pathogen in greater numbers than cankers exposed to ambient conditions. Also that E. amylovora was recovered from a higher percentage of cankered trees grown at 21°C compared to trees held at 17 and 28°C.

Cankers examined throughout the year by Ritchie and Klos showed a sharp decline of E. amylovora populations and the number of active cankers in late fall and early winter. In this study there was no significant difference in canker size and presence of E. amylovora.

Klos stated that E. amylovora frequencies were studied in pear cankers during the dormant season. Maximum frequency occurred at .5-7.5 cm. above the lower margin of the canker and only 1-2 cm. into live tissue. Erwinia herbicola was associated with E. amylovora in those cankers.

NURSERY STOCK

Klos indicated there have been several cases where nursery stock has introduced the disease in new fruit areas. Only clean nursery stock grown in isolated locations should be approved for sale.

BIRDS

In North America the role of birds has not been resolved in dissemination of E. amylovora.

LONG DISTANCE SPREAD

The spread of E. amylovora from North America to England has been speculated to be due to contaminated fruit boxes and/or nursery stock.

The spread from England to the northern regions of the European continent is suspected to be by wind (aerial strands) and birds. The aerial strand theory has not been substantiated. Danish workers indicated that spread could be accomplished during migrating flights of starlings. Common roosting sites in these areas are susceptible *Craetagus* spp. They showed that the bacteria lasted 8 days on artificially infested feet. Flying time from England to Denmark is 2-3 days. This circumstantial evidence indicates long distance spread by migratory birds is a distinct possibility.

BACTERIOPHAGES

A number of researchers in the past have worked with E. amylovora phages mainly as a sensitivity test for the bacteria. Recently, Erskine suggested phages play a role in E. amylovora epidemiology. All his work was done with phages isolated from the soil. Ritchie stated he recently isolated phages from aerial portions of apples. Gibbins reported on a temperate phage of E. herbicola he isolated after treatment with Mitomycin C.

Ritchie is in characterizing E. amylovora phages to determine their role in the etiology of the pathogen.

ERWINIA HERBICOLA

Erwinia herbicola often associated with E. amylovora in some tissues poses as an interesting approach to biological control. Klos indicated that in controlled experiments E. herbicola, when introduced before E. amylovora in blossoms, give up to 50% control. Schroth indicated that his group has research underway studying the use of non-pathogenic bacteria to control pathogenic bacteria. Klos' group is continuing research on the role of E. herbicola as a possible antagonistic agent against E. amylovora.

DISSEMINATION

Spread of fire blight bacteria can be divided in two categories

1. short distance, 2. long distance. Generally we are concerned with short distance which includes insects, wind and rain (storms), strands, man, birds and nursery stock.

INSECTS

Burrill first reported the role of insects in fire blight spread. The importance of insect dissemination will vary from area to another or one orchard to another. California workers feel insects play an important role in dissemination. Other workers have shown that other means are as important if not more important.

There is no doubt insects play an important role particularly in short distance spread. Flowering visiting insects can spread bacteria from flower to flower, tree to tree and even orchard to orchard. The same can be said to sap feeding insects as leaf hoppers and aphids.

WIND AND RAIN

All workers agree that E. amylovora is spread by wind and rain. Recent work in England by Billing indicated the importance of certain types of storms in disseminating bacteria.

STRANDS

Bacterial strands have been recognized in the past as agents of dissemination. Kiel has shown that an agricultural oil increased the production of strands on treated trees.

MAN

Man can spread the pathogen by several means - by contaminate pruning tools, fruit boxes, and ladders. Eradication by summer pruning has to be executed judiciously or more damage will be done than good by actually spreading the pathogen by pruning tools.

PHYSIOLOGY

BIOLOGICAL ACTIVITIES OF THE COMPONENT FRACTIONS OF AMYLOVORIN

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Ooze recovered from slices of immature apple fruit inoculated with a virulent isolate of Erwinia amylovora contains the host-specific toxin, amylovorin. Immediate ethanol precipitation and lyophilization of the ooze upon collection stabilizes the toxin and precludes loss of biological activity (wilt-inducing capacity). The ethanol precipitate was subsequently separated into three fractions on a Dowex 1-X8 column in the carbonate form. The first 2 fractions were eluted with 10^{-2} M and 1M NaHCO_3 with 10^{-3} M EDTA (trisodium salt). The third fraction was eluted with 3M NaCl, 10^{-2} M NaHCO_3 with 10^{-3} M EDTA. Each fraction was dialyzed against 10^{-3} M potassium phosphate, pH 7.0, and lyophilized. Inactive toxin recovered total biological activity when passed through the column. Each fraction contained a carbohydrate and a protein component. The 10^{-2} M fraction contained $\approx 50\mu\text{g}$ of carbohydrate and $\approx 35\mu\text{g}$ of protein per $100\mu\text{g}$. The 1M and 3M fractions contained $\approx 40\mu\text{g}$ and $\approx 20\mu\text{g}$ of carbohydrate and $\approx 30\mu\text{g}$ and $\approx 20\mu\text{g}$ of protein, respectively per $100\mu\text{g}$. Succulent apple shoots, var. Jonathan exhibited strong wilt in 60-90 min. when placed in a $100\mu\text{g}/\text{ml}$ solution of the 3M fraction. Apple shoots placed in $100\mu\text{g}/\text{ml}$ solutions of either the 10^{-2} M or 1M fractions wilted in 90-120 min. Tests of the wilt-inducing capacity of the carbohydrate and protein components of the 10^{-2} M fraction separately, revealed that only the carbohydrate induced wilt. No toxin activity was detected at pH 4.0, 5.0; it was very slight at 6.0; and maximum at pH 7.0 - 8.0.

PRODUCTION OF AMYLOVORIN AND AN AGGLUTINATING FACTOR IN APPLE CELL

SUSPENSION CULTURES INOCULATED WITH ERWINIA AMYLOVORA

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Virulent strains of Erwinia amylovora produce a host-specific toxin, amylovorin, on immature apple fruit tissues, but not in the artificial medium. Studies were performed to determine whether the toxin could be produced in apple cell suspension cultures inoculated with the pathogen. Suspension cultures (apple var. Antonovka) were grown in an agitated liquid medium and inoculated at the late exponential growth phase (14 days) with a virulent E. amylovora strain. Culture filtrates were tested for toxin activity (wilt induction) by the excised Jonathan shoot or leaf bioassay procedures. Toxin activity was detectable 2 days after inoculation with the bacterium and increased over a period of 7 days. The toxin was not detected in sterile culture medium, filtrate from control suspension culture nor filtrates from suspension cultures inoculated with an avirulent strain of the pathogen. The toxin-containing filtrate or partially purified toxin induced wilting of susceptible host but not of nonhost plants. A factor which agglutinated the avirulent strain of the pathogen in vitro also was produced in suspension cultures inoculated with the virulent strain of E. amylovora. Production of the toxin in cultures occurred earlier than that of the agglutinating factor. Separation of the toxin and agglutinin and the biochemical nature of the latter is discussed.

ULTRASTRUCTURAL OBSERVATIONS OF XYLEM-PARENCHYMA DAMAGE AND

XYLEM-VESSEL PLUGGING CAUSED BY THE TOXIN AMYLOVORIN

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Apple shoots var. Jonathan, approx. 10 cm in length, were placed in a solution containing 100 $\mu\text{g/ml}$ of the fireblight toxin amylovorin. Within 2 hours the toxin, which is produced by Erwinia amylovora only in the presence of host cells, caused plasmalemma discontinuities in xylem parenchyma cells. In addition, some xylem parenchyma cells plasmolyzed and neighboring vessels became occluded with a loose gel-like substance. The latter appears in high magnification to be a network of fibrils and granules. The plugging substance accumulates initially between the plasmalemma and wall of xylem parenchyma cells. There is clear evidence that this material then passes from xylem parenchyma into xylem vessels through pits. In addition, some electron micrographs suggest trans-wall passage of the xylem-plugging substance. No ultrastructural distortion was observed in phloem or phloem associated cells. The induction vessel plugging as an active and host specific process is to be discussed.

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FACTORS AFFECTING THE SENSITIVITY OF
ROSACEOUS SHOOTS TO AMYLOVORIN-INDUCED WILT

Thomas M. Sjulín and Steven V. Beer

Assistant Extension Plant Pathologist, University of Illinois, Urbana,
and Assistant Professor, Department of Plant Pathology, Cornell University,
Ithaca, NY, respectively.

Goodman, Huang and Huang [Science 183:1081-1082 (1974)] isolated from fire blight ooze a high molecular weight polysaccharide, amylovorin, which was characterized as a host-specific phytotoxin. When shoots of rosaceous plants were placed in dilute amylovorin solutions, they wilted in a period of time that was negatively correlated with their observed field susceptibility to Erwinia amylovora. Shoots of six non-rosaceous species tested did not wilt in amylovorin solutions.

Studies by Sjulín and Beer [Proc. Amer. Phytopathol. Soc. 2:107 (1976) and 1976 APS Meeting Abstract] indicated that wilt induced by amylovorin in cut shoots of Cotoneaster pannosa was not related to wilt observed in infected shoots. Water potentials of amylovorin-wilted shoots were significantly lower than those of non-wilted shoots. Dextrans of comparable size also induced similar decreases in water potentials. Uptake and transpiration rates of shoots in amylovorin solutions decreased prior to wilt. Flow rates of water in stem segments from amylovorin-wilted shoots were significantly lower than flow rates in segments from non-wilted plants. Electrolyte loss from amylovorin-wilted shoots was not different than that from nonwilted shoots. It was concluded that amylovorin induced wilt by restriction of water movement.

In contrast, the wilt observed in infected shoots does not appear to result from restriction of water movement. Instead, the observed increases in water potential and electrolyte loss in infected tissues suggest that wilt is due to destruction of membrane semi-permeability.

Studies with clonally propagated rosaceous shoots indicated great variability

in the time required for wilt in amylovorin solutions. This report describes attempts to determine factors influencing the sensitivity of rosaceous shoots to wilt induced by amylovorin.

The sensitivity of vegetative shoots of Spiraea vanhouttei and Cotoneaster pannosa to amylovorin-induced wilt was compared with their susceptibility to E. amylovora following artificial inoculation. No infections were observed in shoots of S. vanhouttei inoculated singly with three different isolates of E. amylovora. Inoculation of C. pannosa shoots with the same isolates consistently resulted in typical symptoms of infection. However, no significant difference in the mean time required for wilt in amylovorin solutions (0.5 mg/ml) was observed between S. vanhouttei shoots (53 min) and C. pannosa shoots (78 min).

It appeared that relative succulence of C. pannosa shoots was related to sensitivity to wilt in amylovorin solutions. Succulence of C. pannosa shoots was estimated by measuring the flexibility of each shoot. Flexibility was determined by suspending a 3.5 g weight from the apices of vertically-positioned shoots. The distance in centimeters (to the nearest 0.5 cm) from the shoot apex to the top of the curvature induced by the weight was designated as the succulence index.

A highly significant negative correlation ($P = 0.01$) was observed between the succulence index and the time required for wilt in amylovorin solutions (1.0 mg/ml) for C. pannosa shoots. Although considerable variability could not be explained on the basis of the succulence index ($R^2 = 0.42$), shoot succulence would appear to be an important factor to control during host specificity studies with amylovorin.

Discussion on Amylovorin

Goodman: I would like to have that picture back that shows the ranking system of the amylovorin test. The reason I mention this is because there is a little bit of a hint of how to improve the type of shoot that we can take. There seems to be an unusual example, Steve (Beer), in your system. Steve (Beer) went to great lengths to establish uniformity in shoots. I think that the turgor of the shoot, or its bendability, is perhaps not as good as it might be. Maybe this is a detail but I'll tell you what I've been able to determine. In looking at diffusive resistance of either leaves or shoots, I find that I can tell in a group of 10 shoots of the same variety, let's say Red Delicious or Jonathan, those which will respond to toxin quickly and those which will not. Which means something that those who have used the test have discovered, that shoots are not uniform in their response to toxin. There is in fact a great deal of variability. But I have been able to take a group of ten and quickly establish those which are responders and those which are not. And what I've done is measure diffusive resistance. I can take a shoot like this and while it's in water (its base has been cut) I can measure diffusive resistance and get a deflection (on the meter). If it's a rapid one, I know that once put in toxin, it will go down (wilt) in a very short period of time. If I take another one at random, exhibiting increasing diffusive resistance, that particular shoot will not take up toxin. My theory is this: That shoots vary in the rate at which they can pick up toxin. I think that the system itself is terribly important. If you went out to the field yesterday and saw what pains are taken in putting out varieties and the possible loss of an entire block by inoculation, there is some merit in perfecting a system like this if, in fact, it is perfected. I repeat this, if, in fact, it is perfected. I believe that, with careful monitoring of half-a-dozen shoots, or ten shoots for that matter, I can pick out now three or four shoots that I am sure will respond very vigorously to the toxin, again indicating that all shoots are not equally capable of reacting at the same speed or to the same intensity. And so, if you do a test, and

this test was done really very effectively, very scientifically, and I think, all precautions were taken to give both the toxins (amylovorin preparations) a fair test, however, I believe that, knowing what I know, four shoots are taken using the technique of selection that Steve (Beer) used is not, perhaps good enough.

Beer: Pardon me, Bob, I didn't select them.

Goodman: No, you selected them, or how did they come into your hands?

Beer: We had, what was it, about 30 potted plants of each cultivar, and we measured flexibility.

Goodman: Yes, OK, that's the selection procedure.

Beer: Right.

Goodman: I was very careful to say that you used proper techniques. I'm not going to say anything disparaging about the way you pick your shoots. I'm just saying that there is, or may well be, a better way to select shoots for sensitivity to the toxin. My final point is, I believe that at least in my hands, each time I try and do it, we use ten shoots at random, without this preselection, the difference between great resistance (Red Delicious) and Jonathan is so clear that there is at least a 6 to 8 hour difference in the time that wilt occurs. Before we get something like this, you can get something in Jonathan that occurs in 2 hours here and takes 12 hours, or longer to get with Red Delicious. I do think, of course, to give this technique as it now has been developed to people like Herb (Aldwinckle) or Gordon Bonn, it's probably not ready for that sort of thing. But I do believe that it has a degree of merit. I can't believe that what I see is a figment of my imagination and I think also it's a matter of time before we are able to establish whether there is fact or isn't.

Aldwinckle: I wonder whether other people have had experiences similar to those of Bob (Goodman) or Steve (Beer). We haven't had any kind of evidence on pears, for instance. I think in your (Goodman's) original Science paper you had more pear varieties

than apple varieties. So this might be something worth looking into.

Bonn: I haven't been able to do too much work, but what I have done is to test pear vs. a number of Prunus species and I find that there is a marked difference. The pears wilt but the Prunus spp. do not. Where I have run into problems is in the variability of my plant material. And this is why you have to find, or select, material that will not be variable.

Goodman: I really feel that there is an inherent way to judge the sensitivity of a given apple or pear shoot to the toxin itself. I think you (Beer) have judged that the importance of light is a significant one in the wilt test. Which means that so little efficiency, or so little aperture, is terribly important in this process. Maybe we are not paying attention, for example, to shoots that are shade shoots, shoots that perhaps don't have the correct stomatal aperture. I believe that using a tool that actually gives you the amount of water being transpired in control shoot before it's been treated, allows you to assess just how efficiently or--how much transpiration is taking place from a leaf surface. Then I am able to pick out shoots that I know will go down very quickly to toxin.

Beer: I wouldn't argue with that. But I'm saying that the conditions that we used were I think, as uniform as one could hope to get in selecting new material. It was clonally propagated material grown in the greenhouse. We did get differences in the mean wilt indexes of the various cultivars. There seems to be a cultivar effect. Some of these varieties that we used are not commonly planted, not that well known, but others of them are. If we forget for a moment that we knew what these cultivars were and consider that they are selections from breeding programs, all clonally propagated, based on amylovorin sensitivity, we would have judged Prima as susceptible, and York as resistant. From Herb's (Aldwinckle) tests, and our own field observations, we know that the reverse is true. So we'd be throwing out the resistant and keeping the susceptible.

Goodman: Well, I would hope that you wouldn't press any kind of a screening system that quickly, and throw away breeding material based on something that's been extant for about a year. No one in my laboratory has suggested that this is the way it is to be done. We haven't said that this system should be used, must be used, or can be used successfully. It has to be tried, and if it's found wanting, either forget it or try to modify it.

Van der Zwet: Just one comment. In our pear breeding program we had used Bob's (Goodman) amylovorin; it was two years ago, when Regine Samson came right from your (Goodman's) lab to our lab. We were unable to show what you had published. However, in one minor test, a bunch of seedlings from a very susceptible cross did react. Due to the Handbook, we haven't been able to get to it, but, next year I want to get together with you (Goodman) and test amylovorin against our pears.

Goodman: One final thing, we're now very happy to supply this material for many people to test and if I felt that there was not much of a possibility or I wanted to substantiate my own results personally, I don't think I'd be that free with the material that I'm sending out. I expected it to be tested. If it's no good you'll know about it. If my current theory is one that won't stand the test of time, it's not terribly important.

Aldwinckle: One small point I'd like to make myself. I was interested in the pears as against the apples because in your Science paper and in subsequent discussions you've mentioned two apple varieties, Jonathan and Red Delicious, one susceptible and one resistant, and the susceptible wilts quicker than the resistant. So if we looked at our list of nine cultivars, which Steve (Beer) showed, we could pick out one susceptible and one resistant and we could find the susceptible wilting faster than the resistant, but that's only two and it doesn't hold up.

Goodman: It may be fortuitous, you say?

Aldwinckle: Yes, whereas there were more pears in your Science paper, so I was wondering about that.

Kuc: I think it's important not to confuse the role of amylovorin in the host-parasite interaction with its use as a mechanism for screening. I can very easily see it having a very important role in the host-parasite interaction, if not the key, certainly one of the very principal mechanisms for susceptibility and yet the technique itself that's used for cutting the shoot having exceptions. I might add that we also were interested in this, in amylovorin before we knew it was amylovorin. We had a graduate student working on the problem. Bob (Goodman) spoke to me, or somehow I learned of the progress that he had made before the publication and Bob (Goodman) very graciously gave us information about isolation, etc., of the material and we had tested it with Delicious and Jonathan. The Delicious was resistant, the Jonathan, very susceptible (to wilt) with that shoot test. We tested some pear varieties, we had one notable exception in our pear varieties. We have a numbered line, which is essentially immune (to E. amylovora) but in our hands wilts very rapidly. But I think that this is a character of cutting that shoot and it's very sensitive. So I think there are other factors which superimpose upon the sensitivity to the toxin and I think cutting the shoot and its inherent ability to wilt is such a complication. But I don't think that detracts from the role of amylovorin in the host-parasite interaction.

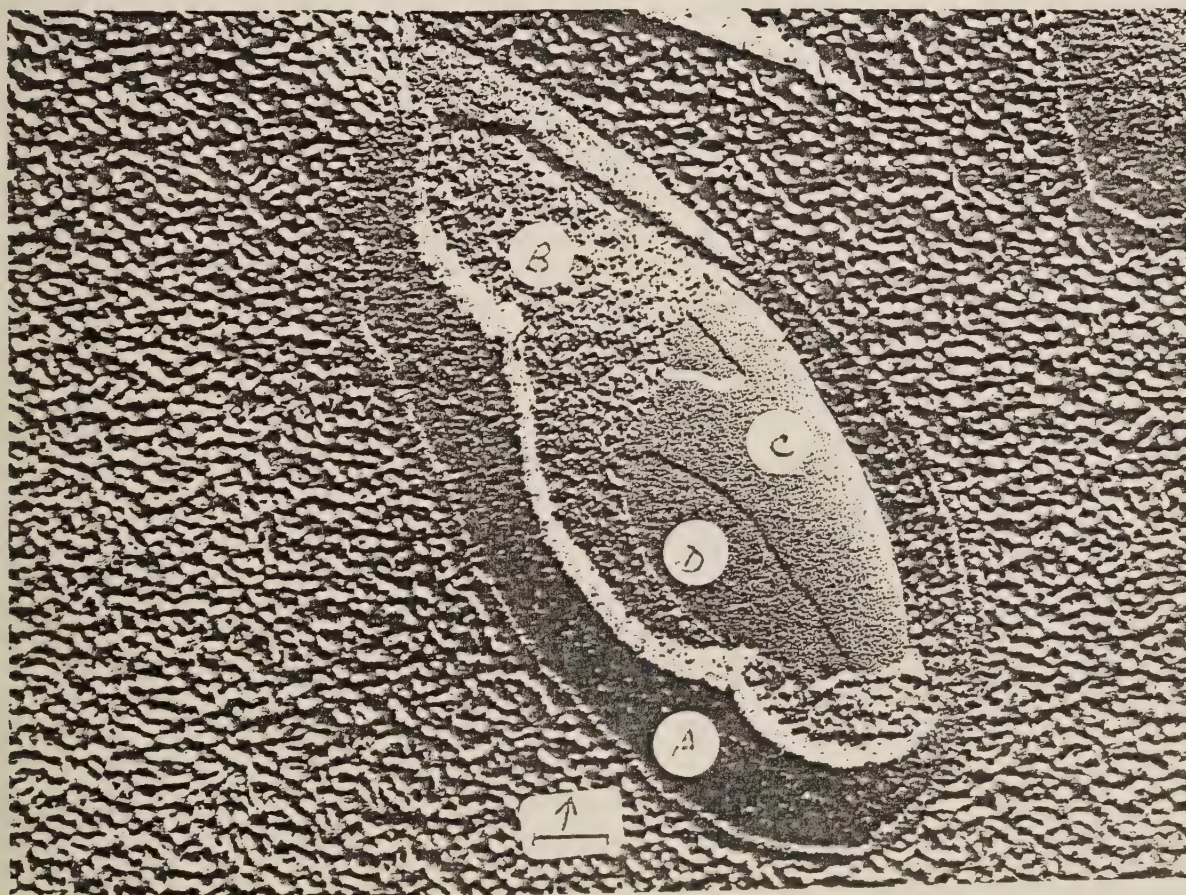
"A study of the ultrastructure of the cell envelope of *Erwinia amylovora* NCPPB595 using the freeze-fracture technique."

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The fracture planes exposed by the freeze fracture technique in the outer regions of the cell envelope of *Erwinia amylovora* NCPPB595, grown to exponential or stationary phases in yeast beef broth at 30°C, confirmed the structure typical of Gram-negative bacteria. At the level of the plasma membrane, however, a hitherto undescribed mode of fracture, which exposed an additional plane, was observed. The explanation of this plane required a re-evaluation of the current interpretation of the location of freeze-fracture planes at the level of the plasma membrane in Gram-negative organisms. It is proposed that, in order for cleavage to occur through the hydrophobic interior of the plasma membrane of *E. amylovora* NCPPB595, the membrane at the site of fracture must be devoid of included particles.

This work was supported by the National Research Council of Canada.



A: convex surface exposed by probable cleavage of the outer membrane; B: outer surface of the cytoplasmic membrane (particulate); C: outer surface of the cytoplasmic membrane (non-particulate); D: surface revealed by cleavage of the cytoplasmic membrane. The arrow indicates the direction of metal deposition, and the bar represents 0.1 nm.

Physiology of virulence in Erwinia amylovora.

- I. Barrier property of the E. amylovora cell envelope in relation to plant virulence.
- II. Contribution of the gal operon of E. amylovora to plant virulence

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The mechanism(s) by which Erwinia amylovora produces the symptoms of fire-blight disease in apples and pears is at present unclear. Goodman and his associates (Goodman and Huang, 1974; Hsu and Goodman, 1976) have reported that a polygalactose toxin, amylovorin, is produced by the bacteria in the susceptible host plant or plant parts but not in culture media. There is, however, some controversy regarding the toxic role of amylovorin. Sjulín and Beer (1976) have demonstrated that the infection of the host by Erwinia amylovora caused an alteration of cell permeability, whereas amylovorin caused wilting of the host plants by a non-specific restriction of water movement. These observations cast doubt on the alleged specific role of amylovorin in the fire-blight disease (Goodman and Huang, 1974). The possibility remains that the bacterium might produce in the susceptible plant host a toxin (in addition to amylovorin) which is host-specific and alters host cell permeability, eventually leading to host cell death. If this notion is correct, then the two processes--i.e., production of necrosis by a specific necrotoxin, and production of ooze (\equiv or \neq to amylovorin; polygalactose)--should be separable. One way of effecting this separation is by mutant analysis. The isolation of E. amylovora mutants which produce necrosis of the host but produce very little or no ooze might implicate the role of a toxin other than amylovorin in the fire-blight syndrome. We have now obtained mutants of E. amylovora which produce necrosis when inoculated onto the skins of immature pear fruits, but which do not produce ooze when inoculated onto the cut surfaces of such pears. The properties of some of these mutants are described below.

We have taken an additional approach in determining the role of amylovorin in the fire-blight disease. Analysis of the purified polysaccharide portion of the amylovorin has revealed that 70 per cent (by weight) is made up of galactose (Goodman et al., 1974; Eden-Green and Knee, 1974; Seemüller and Beer, 1976), and the molecule has therefore been referred to as "polygalactose". Further, Stoffl, Karr, and Goodman (1976) have shown that only the carbohydrate moiety of the toxin molecule induced wilting of Jonathan apple shoots. The genes controlling polygalactose production are probably located on the E. amylovora chromosome. Production of

polygalactose might occur via formation of nucleotide-galactose (probably as UDP-galactose) and the subsequent transfer of galactose from UDP-galactose to the nascent polygalactose chain by a specific transferase activity. If this assumption is correct, simultaneous blocks in the entry and activation of galactose--i.e., in the formation of gal-1-P (gal K) and UDP-galactose (gal T) and in the conversion of UDP-glucose to UDP-galactose (gal E) [Fig. 1a] or a block in the transferase activity--would prevent the synthesis of polygalactose by the bacterium. In both E. coli and S. typhimurium, the genes (gal OETK) of the gal operon are clustered in one region [Fig. 1b] (Bachmann et al., 1976; Sanderson, 1972; Sanderson and Hartman, in preparation). We have now found a gross similarity in the location of genes on E. amylovora and E. coli or S. typhimurium chromosomes. On this basis, one might postulate that the strains of E. amylovora in which the gal operon is deleted or non-functional might fail to produce the ooze (polygalactose) when inoculated onto immature pear fruits. In addition, studies on such mutants might reveal whether polygalactose production is determined by the gal operon and whether it is required in the pathogenesis of E. amylovora strains. Some of our preliminary results (Chatterjee and Starr, in preparation) with spontaneous gal⁻ mutants of E. amylovora, presented below, reveal that some gal⁻ mutants cause necrosis of immature pear fruits but produce very little or no ooze. Our results also suggest that a functional gal operon is required for full virulence, suggesting a role, perhaps of secondary nature, of amylovorin (polygalactose) in the fire-blight disease.

We (Chatterjee, Buss, and Starr, in preparation) have also found that the wild-type strains of E. amylovora are markedly sensitive to novobiocin, deoxycholate, and sodium dodecyl sulfate. The LD₉₉ (μg/ml) of these three agents were 15-100, 40-800, and 50-800, respectively, depending on the strain of E. amylovora tested [Table 1]. The growth of other Erwinia spp. (E. carotovora and E. herbicola) and of Salmonella typhimurium was not affected at all, or was only slightly affected, at these concentrations of these agents. The presence in E. amylovora strain EA178 of the plasmids F'lac⁺, RPl, and R100drd-56 resulted in enhanced sensitivity of the bacterial strain to novobiocin and sodium dodecyl sulfate; these plasmids did not alter the resistance of S. typhimurium LT2 to the agents [Table 2]. E. amylovora strains spontaneously release into the growth medium a periplasmic enzyme, cyclic phosphodiesterase [Table 3]. The absence of

detectable activity of a cytoplasmic enzyme, glucose-6-phosphate dehydrogenase, in the culture supernate indicates that extensive cell lysis did not occur. Addition of MgCl_2 (20 mM) and sodium chloride (84 mM) to Tryptone broth stimulated the growth of E. amylovora strains and prevented the release of the periplasmic enzyme [Table 3]. The extensive loss of the periplasmic enzyme and sensitivity of the strains to the three antibacterial agents seem to result from defect(s) in some component(s) of the cell envelope other than the lipopolysaccharide of these bacteria. Mutants, obtained by ethyl methane sulfonate treatment of E. amylovora strain EA178, which are resistant to each agent separately (or to all three of them), revealed a correlation between resistance to these agents and periplasmic leakiness [Tables 4 and 5]. The mutants resistant to these agents show a decrease in virulence, i.e., in the extent of necrosis and in the quantity of exudate produced on immature pear fruitlets [Table 6]. These observations suggested to us that a defective cell envelope plays a role in pathogenesis and, hence, has been conserved in these phytopathogenic bacteria.

Virulence of gal⁻ mutants. The results summarized in Tables 7 and 8 reveal that spontaneous gal⁻ mutants of EA213 (E9) and EA198, both virulent strains, exhibit a variety of virulence phenotypes [Table 9]. Based upon these results, the following tentative conclusions can be made regarding the role of the gal operon in virulence of E. amylovora:

- a. A functional gal operon (or, at least, part of it) is necessary for full virulence.
- b. A necrotic reaction can occur in the absence of ooze production.
- c. Ooze production is dependent upon the development of necrosis. In no case have we detected ooze production in the absence of necrosis.
- d. The gal operon of E. amylovora is involved in formation of ooze.

Whether ooze \equiv polygalactose \equiv amylovorin is unclear.

These results lead us to propose the following tentative model for the virulence of E. amylovora:

E. amylovora, once inside the plant tissue, multiplies and produces a toxin (for the moment to be referred to as "necrotoxin") which alters the host cell permeability and eventually causes host cell death. It is possible that the production of this necrotoxin, at least to a limited extent, is necessary for the selective multiplication of the pathogen, in which case the phase of multiplication (increase in cell population) is

followed by a phase of active necrotoxin production and hence of rapid plant cell death. During the later phase of disease, hypothetical activators present in the plant might activate bacterial components to produce polygalactose; the polygalactose thus produced then affects the plant host cells in a non-specific manner. It is possible that the production of polygalactose slime at a later phase of the infection process also provides a means for the bacterial cells to survive in nature under the protective coating of the polymer. This model predicts that polygalactose plays a secondary role in the fire-blight disease; this prediction is consistent with the findings of Sjulín and Beer (1976) and of Eden-Green and Knee (1974).

If polygalactose (amylovorin) is of secondary importance, how does one explain the loss of virulence in some gal⁻ strains? It is possible that galactose and/or the metabolic products of galactose are also components of the "necrotoxin"; if the genes involved in this conversion (galactose → necrotoxin) are defective (or deleted) in the gal⁻ strains, this mechanism would explain the total avirulence of these gal⁻ mutants.

It is equally possible that--in some gal⁻ mutants which are completely avirulent--there is an alteration in the cell surface, perhaps in the lipopolysaccharide molecule present in the outer membrane. This postulated alteration of the cell surface might prevent cellular interaction (including recognition) between plant and bacterium, a process perhaps necessary to initiate the infection process. This latter hypothesis is attractive to us for the following reasons: (a) we know that galactose is present in the lipopolysaccharide molecules of wild-type strains of E. amylovora; and (b) mutant strains of E. amylovora, the cell surfaces of which have been altered (those which are resistant to novobiocin, deoxycholate, and sodium dodecyl sulfate), are relatively less virulent than the wild-type strain (Chatterjee, Buss, and Starr, in preparation). Following such reasoning, a role of the cell surface in the pathogenic process seems to be indicated and, hence, alteration of the cell surface of E. amylovora (by, for example, gal deletion) could result in altered virulence.

It would be premature at this time to indulge in further speculations on the specific role of the gal operon, especially in the absence of data on the mutants with regard to (a) the genetics and enzymology of the gal operon, and (b) the multiplication of the gal⁻ mutants in the host tissue. Studies along these lines are being carried out in our laboratory

(Chatterjee and Starr, in progress), and we expect that the results will establish the nature of the specific contribution(s) of the gal operon in the fire-blight disease.

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TABLE 1.

SENSITIVITY OF BACTERIAL CULTURES TO DEOXYCHOLATE (DOC),
NOVOBIOCIN (NOV), AND SODIUM DODECYLSULFATE (SDS)

SPECIES	LD ₉₉ (μG/ML) WITH		
	NOV	DOC	SDS
ERWINIA AMYLOVORA E8	>50	400-800	200-500
E9	>50	250-500	200-500
EA137			
EA167	15	250-500	40
EA177			
EA178			
ERWINIA HERBICOLA, ERWINIA CAROTOVORA, & SALMONELLA TYPHIMURIUM	>100	>1000	>1000

TABLE 2.

SENSITIVITY OF PLASMID-BEARING STRAINS OF ERWINIA AMYLOVORA
(EA178) TO NOVOBIOCIN (NOV), DEOXYCHOLATE (DOC) AND SODIUM
DODECYL SULFATE (SDS)

<u>PLASMID</u>	LD ₉₉ (μG/ML) WITH		
	<u>NOV</u>	<u>DOC</u>	<u>SDS</u>
NONE	15	250-500	40
F' <u>LAC</u> ⁺	5-10	"	25
R100 <u>DRD</u> -56	5-10	"	25
RPI	5-10	"	25
E- <u>LAC</u> ⁺	15	"	40

TABLE 3.

EFFECTS OF SODIUM CHLORIDE AND MAGNESIUM CHLORIDE ON THE GROWTH
AND SPONTANEOUS RELEASE OF ENZYMES IN ERWINIA AMYLOVORA (EA178)
AT 30C IN TRYPTONE BROTH

SUPPLEMENT	GROWTH (KLETT UNITS)	GENERATION TIME (MIN)	CYCLIC PHOSPHO- DIESTERASE ACTIVITY IN CULTURE SUPER- NATANT (% OF TOTAL)*
NONE	92	120	68
NaCl (84mM)	180	60	17
NaCl (168mM)	160	60	17
MgCl ₂ (20mM)	151	60	NIL

* GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY PRESENT IN CELLS, BUT NOT
IN SUPERNATANTS

TABLE 4.

SENSITIVITY PATTERN OF ERWINIA AMYLOVORA STRAIN EA178
AND THE MUTANTS DERIVED THEREFROM TO NOV, DOC, AND SDS

STRAIN	LD ₉₉ (μg/ML) WITH			OTHER CHARACTERISTICS
	NOV	DOC	SDS	
EA178*	15	250-500	40	Nic ⁻
AC 416 (DOC ^R)	<25	>1000	<250	DERIVED FROM EA178
AC 418 (NOV ^R)	>100	500-1000	<250	DERIVED FROM EA178
AC 419 (SDS ^R)	>100	500-1000	>1000	DERIVED FROM EA178; ALSO ARG ⁻

* EA178 IS A VIRULENT WILD-TYPE STRAIN

TABLE 5.

SPONTANEOUS RELEASE OF PERIPLASMIC AND CYTOPLASMIC ENZYMES
BY ERWINIA AMYLOVORA WILD-TYPE AND DRUG AND DETERGENT
RESISTANT STRAINS IN TRYPTONE BROTH AT 30C

STRAIN	ACTIVITY PRESENT IN CULTURE SUPERNATANT (PER CENT OF TOTAL)	
	CYCLIC PHOSPHODIESTERASE	GLUCOSE-6-PHOSPHATE DEHYDROGENASE
WILD TYPE (E8, E9, EA137, EA167, EA169, EA177, EA178, EA198)	55-70	NIL
AC 416 (DOC ^R)	25	NIL
AC 418 (NOV ^R)	45	NIL
AC 419 (SDS ^R)	35	NIL

TABLE 6.

VIRULENCE OF ERWINIA AMYLOVORA WILD TYPE STRAIN EA178
AND THE DRUG- AND DETERGENT-RESISTANT MUTANTS

STRAIN	REACTION ON PEAR FRUITLETS AFTER 5 DAYS	
	NECROSIS	EXUDATE
EA178	++	+
AC 416 (DOC ^R)	+	NONE
AC 418 (NOV ^R)	+	NONE
AC 419 (SDS ^R)	+	NONE

++ = 3-5 MM BROWNING

+ = 1-2 MM BROWNING

TABLE 7.

VIRULENCE OF GAL- MUTANTS OF E9

STRAIN	VIRULENCE	
	NECROSIS	EXUDATE
E9	+++	++
E9 <u>GAL</u> -1	++	+
E9 <u>GAL</u> -2	+	±
E9 <u>GAL</u> -8	-	-
E9 <u>GAL</u> -20	+	-

NECROSIS:

+++ = 7-10 MM BROWNING IN 5 DAYS

++ = 2-3 " " " " "

+ = 1-2 " " " " "

- = NO NECROSIS

EXUDATE:

++ = COPIOUS OOZE COVERING THE ENTIRE CUT SURFACE

+ = LOCALIZED OOZE PRODUCTION

± = SCATTERED SMALLER GLOBULAR OOZE; PIN-HEAD LIKE

- = NO OOZE PRODUCTION

TABLE 8.

VIRULENCE OF GAL- MUTANTS OF EA198

STRAIN	VIRULENCE	
	NECROSIS	EXUDATE
EA198	+++	++
EA198 GAL-4	+++	++
EA198 GAL-14	±	±
EA198 GAL-18	+++	±

NECROSIS:

+++ = 7-10 MM BROWNING IN 5 DAYS

± = TRACE (≤1MM)

EXUDATE:

++ = COPIOUS OOZE COVERING THE ENTIRE CUT SURFACE

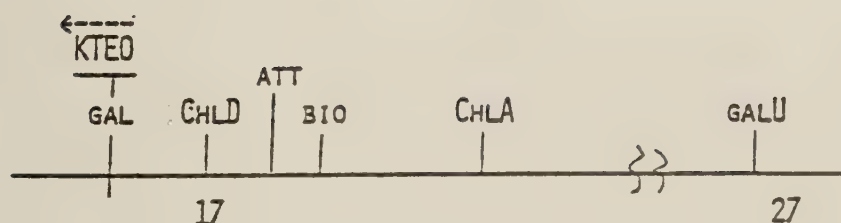
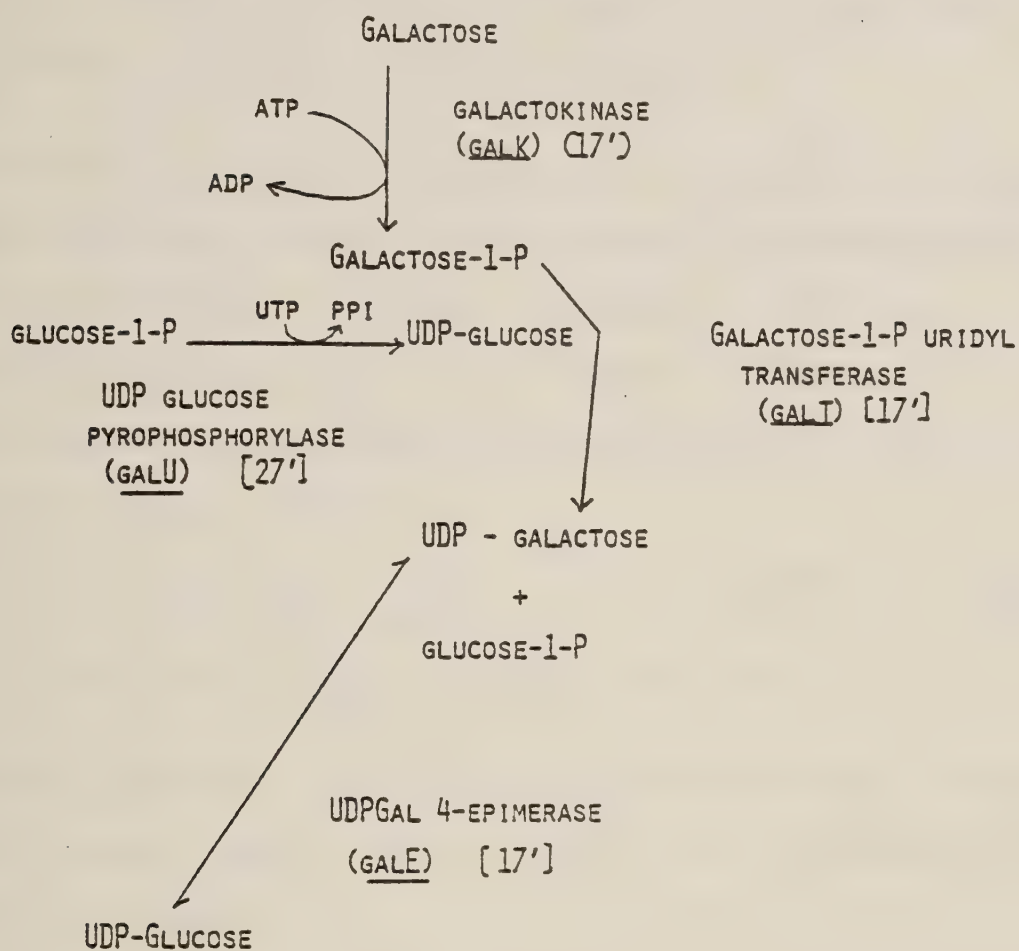
± = SCATTERED SMALLER GLOBULAR OOZE; PIN-HEAD LIKE

TABLE 9.

VIRULENCE PHENOTYPES (CLASSES) OF GAL- MUTANTS OF E9 AND EA198

- I. NECROSIS AND OOZE PRODUCTION LIKE WILD TYPE
- II. DECREASED NECROSIS AND OOZE PRODUCTION
- III. DECREASED NECROSIS AND VERY SLIGHT OOZE
- IV. DECREASED NECROSIS, NO OOZE
- V. WILD TYPE NECROSIS, VERY SLIGHT OOZE
- VI. NO NECROSIS, NO OOZE PRODUCTION

FIGURE 1a. Pathways of UDP-galactose synthesis in enterobacteria.

FIGURE 1b. GAL REGION OF E. COLI K12

Pertinent Statements Made During the Discussion on Physiology (Part I)

I venture to guess that E. amylovora has more than one metabolite which it produces that causes the complete syndrome of the disease and that the polysaccharide is the key to infection. (Goodman)

Succulent shoots of Cotoneaster pannosa were inoculated with E. amylovora 7 cm below the apex; even though ooze production was observed after 5 days on the inoculated shoots, there was no indication of difference in water potential and no wilting occurred; this indicated that his restriction in water movement we see with amylovorin did not occur to any great extent in the infected tissues. (Sjulin)

The phenomenon studied in C. pannosa does not appear to be analogous with observed results in Malus and Pyrus. (McIntyre)

The virulent strain of E. amylovora constantly produces the polysaccharide but the avirulent strain is unable to produce this polysaccharide that is active. (Goodman)

Biochemical Changes and Induced Resistance in the Interaction of
Pear or Apple with Erwinia Amylovora

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It has been well established in the literature by the studies of Hildebrand, Schroth, Smale, Kuc, Raa, Podstolski and their colleagues that both pear and apple contain glycosides which, upon hydrolysis and oxidation of the liberated aglycone, or direct oxidation of the glycoside, yield fungistatic and bacteriostatic compounds. The glycoside arbutin in pear and phloridzin in apple foliage are the major glycosides, and after injury or infection resulting in injury, the glycosides are decompartmentalized in the cell, hydrolyzed by β -glycosidases and the glycosides or their aglycones oxidized by phenoloxidases and peroxidases. The production of this antibiotic activity is certainly non-specific and is dependent upon injury. Varietal resistance and susceptibility to fire blight does not appear to be explained on the basis of β -glycosidase, peroxidase or phenoloxidase activity or the concentration of the glycosides in the tissues. Nevertheless, the antibiotic activity these compounds generate cannot be ignored and may function as part of a multicomponent mechanism for general disease resistance - perhaps a repair mechanism in the tissue.

Indications are that a degree of specificity exists in the interaction of pear or apple cultivars with E. amylovora. The pathogen is largely limited to hosts in the Rosaceae. There is a need for a critical concentration of inoculum for infection and the mean temperature is important in determining symptom development. Stress conditions (wind and hailstorms)

often precede severe outbreaks of fire blight, and these stress conditions may do more than provide wounds for the penetration of the bacterium. Suggestions have been made that the stress affects the metabolism of the host to enhance development of a limited and controlled population of the pathogen present in the host.

Recent studies in our laboratory have indicated that an effective mechanism for resistance to fire blight can be elicited in tissues without implicating glycosides and their hydrolysis and oxidation. The mechanism does not principally affect multiplication of the pathogen in the host, but rather affects the expression of symptoms. This raises several considerations:

- (1) All varieties of pear and apple have the potential for highly effective mechanisms for resistance to fire blight if these mechanisms can be activated soon enough and with sufficient magnitude.
- (2) E. amylovora can multiply in host tissues without causing symptoms; therefore, the two processes may be under different metabolic and environmental controls.
- (3) Resistance to fire blight may be determined by at least two different mechanisms. One mechanism limiting development of the bacterium and the other the expression of symptoms.

The mechanisms could operate to permit extensive bacterial multiplication with limited disease expression or extensive disease expression from limited bacterial multiplication. The ability to activate the mechanisms with time may explain the restriction of canker development on highly susceptible hosts. A discussion of our work on the protection of apple and pear against fire blight using bacterial DNA follows.

Protection of Pear Against Fire Blight with
Deoxyribonucleic Acid from Erwinia Amylovora

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Protection of 'Bartlett' pear and 'Jonathan' apple against fire blight, incited by E. amylovora, has been reported with avirulent E. amylovora, E. herbicola, and Pseudomonas tabaci (1, 2, 4). Recently it was demonstrated that cell-free sonicates of avirulent and virulent E. amylovora protect against fire blight (2). The sonicates were not inhibitory to the pathogen and did not alter its virulence. Further investigations (3) demonstrated that DNA from virulent or avirulent E. amylovora is the active protectant.

Sonicates of E. amylovora protected the shoots of young trees, etiolated and green germinated seedlings of pear, but protection was lost when nucleic acids were precipitated with protamine sulfate. The growth of virulent E. amylovora in protected and unprotected etiolated seedlings was approximately equal. Nucleic acids purified from sonicates protected, and treatment with DNase but not with RNase destroyed activity. The nucleic acids isolated from sonicates were separated into two 258 nm absorbing peaks by linear log sucrose gradients. The two peaks were found to be RNA and DNA by treatment of nucleic acid prep-

arations with DNase or RNase prior to centrifugation. DNA reisolated from the sucrose gradients protected as did DNA isolated by the Marmur technique. Protection of etiolated seedlings against fire blight was found to depend on DNA concentration and DNA did not affect the growth of E. amylovora in vitro. E. amylovora remained virulent when grown in culture with DNA from the avirulent bacterium. Melting curves indicated the DNA had a relatively high molecular weight. Isolated DNA from cesium chloride centrifugation also protected against fire blight, and apple seedlings were protected by DNA from the virulent and avirulent bacterium.

DNA was infused into seedlings from which the radicle had been excised and the seedlings were subsequently often observed to form roots. Rooted seedlings were transplanted into vermiculite and soil and kept between baking dishes containing wet filter paper. Seedlings maintained in this manner for one week were challenged 0.5 cm below the cotyledons. Control seedlings showed fire blight symptoms within two days of challenge, whereas seedlings protected with DNA showed no symptoms for at least one week. This demonstrates the persistence of protection with DNA and that protection occurs even at sites removed from the point of initial DNA application.

Lack of protection by DNA from Bartlett pear seedlings, bovine serum albumin, RNA from E. amylovora, apoferritin, polypeptides, polyanions, polycations, salmon sperm DNA, nitrogen bases, ribose, deoxyribose, nucleosides and nucleotides indicates that protection is not directly related to size or charge. Increased protection by relatively unsheared DNA (Marmur technique) suggests some relationship between native composition of the DNA and protection. Protection of pear with

DNA from virulent E. amylovora suggests that the virulent bacterium initiates interactions with the host which are responsible for symptom expression before its DNA can protect. Protection requires a time period between injection of DNA and challenge. Aside from the possibilities for the control of fire blight, the work with DNA and protection also raises some questions concerning specificity. In protected tissue the virulent pathogen multiplies but does not cause disease. E. herbicola and avirulent E. amylovora also multiply in apple and pear, do not cause disease, and protect against virulent E. amylovora. Specificity in these instances is not dependent solely upon whether the bacterium multiplies.

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Effect of Erwinia amylovora DNA on Fire Blight Resistance of Bartlett Pear

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DNA extracted from Erwinia amylovora by Kuc and coworkers was used in various greenhouse studies at Beltsville in an attempt to increase fire blight resistance of Bartlett pear. Four tests were conducted as follows:

1) Injected DNA solution into pear shoots (2'-3') through tip $\frac{1}{2}$ inch from apex with hypodermic needle. A total of 1 ml (200 μ g/ml) of DNA solution was injected into 10 shoots. Challenged 24 hours later with E. amylovora (10^5 cells/ml). Each treatment replicated 10 times. Fourteen days later, the number of shoots blighted and the depth of penetration was recorded as follows:

Treatment	Number shoots blighted	Average blight penetration (inches)
Check	9	7.4
tris HCl buffer (0.05M) ph 7.5	10	8.4
DNA (200 μ g/ml)	9	8.2

2) Potted Bartlett seedlings 2 feet tall were cut back to 7 inches. Each plant had 11-16 leaves remaining on the plant at this time. Roots were washed free of soil and then placed in test solution for 24 hours then transplanted into 6" pots containing 50% soil plus 50% Jiffy mix (sphagnum + vermiculite). Inoculated the secondary shoots (1"-7") developing from the axillary buds with E. amylovora (10^5 cells/ml) through a hypodermic needle. Each treatment was replicated 10 times. The following results were obtained.

Treatment	No. ml solution/beaker	Average amount solution taken up/plant (ml)	No. shoots blighted
Phosphate buffer	200	28.4	25/27
DNA buffer (10 μ g/ml)	200	23.0	16/16

3) Bud sticks 6 inches long ($\frac{1}{4}$ " diameter) were placed with their basal portion in the test solution and a rubber hose placed over the opposite end. The hose was connected to an air suction system and the test solution pulled through the bud sticks. Pulled 10 ml of DNA solution (200 μ g/ml) through a total of 10 bud sticks. Buds from these scions were grafted into Bartlett

pear seedlings. When these buds produced shoots 2-8 inches long, they were inoculated with E. amylovora (10^5 cells/ml). The following results were obtained:

Treatment	No. shoots blighted
Buffer	9/9
DNA-buffer (200 μ g/ml)	10/10

4) Bartlett pear whips were cut back to 6 inches and all leaves removed. When the axillary buds started to break, a penicillin assay disc (12.7 mm) with a small hole in the middle was placed over the tip of the bud. Ten drops of test solution was placed on each disc 2 times. Those buds which developed into shoots (2"-5") were inoculated with E. amylovora (10^5 cells/ml). The following results were obtained:

Treatment	No. plants with new shoots	No. blighted shoots
Buffer	4	all
DNA (10 μ g/ml)	1	all
DNA (100 μ g/ml)	2	all
DNA (200 μ g/ml)	3	all

Under conditions of these tests, we conclude that the DNA solution failed to induce any degree of fire blight resistance in Bartlett pear plants.

Effect of Leaf pH on Fire Blight Resistance
Harry L. Keil ^{1/}

In studies at Beltsville, periodic leaf pH readings were made in 1967 and 1968 on several field-grown pear cultivars and Jonathan apple to see whether any significant changes took place during the season and how pH could be assimilated into the fire blight syndrome. One gram of either old or young leaves was added to 50 ml of demineralized water and thoroughly chopped and mixed with a Waring blender. The pH readings were made on the resulting leaf-water suspension. Young leaves up to three-fourths grown of all seven pear cultivars-Bartlett, Stewart Bartlett, Dawn, DeVoe, Kieffer, Magness, and Moonglow-showed a striking increase in acidity from April 24 to June 13. On April 24 (full bloom to petal fall) all cultivars showed relatively high pH values (5.3-6.0). Such values are known to support optimum growth of E. amylovora in vitro (1, 2, 3, 4, 5). On subsequent test dates (May 8 and June 13) the pH values of young leaves progressively decreased. Young leaves of Bartlett and Stewart Bartlett, both highly susceptible cultivars, had pH values never below 5.2 at any time during the study, whereas Magness, Kieffer, and Moonglow had pH values of 4.6, 4.9 and 4.9, respectively. No such increase in acidity was observed in Jonathan apple. As pear leaves became older, the pH values appeared to stabilize and showed values less acid than young leaves sampled on the same date.

Based on these results, one may argue that the old leaves support growth of the fire blight organism more than young leaves. However, this is probably unlikely because other factors, such as morphology and phenolic potential, take over as leaves mature. It is therefore

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suspected that the pH of the sap of pear cultivars may in part affect the resistance of susceptible young leaves of a given cultivar. Further studies comparing young leaves and their petioles demonstrated that the latter always had higher pH values than the former. This appeared to correlate with our field observations and greenhouse inoculation studies. Studies also indicated lower pH values of leaves and petioles on resistant Magness and Moonglow cultivars compared with higher values for those on the susceptible Bartlett. Because some of the cultivars acted similarly in the field, these data indicate that additional studies should be conducted to resolve the effect of sap pH on fire blight resistance.

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Pertinent Statements Made During Discussion on Physiology (Part II)

It is possible that entire DNA is broken down into nucleotides and then reassembled into plant DNA. However, one experiment makes one stop and think of the possible movement of DNA through plants:

Methods: introduced labelled ("hot") DNA into seedlings with decapitated roots; then put seedlings into water or unlabelled ("cold") DNA to determine concentration gradient.

Results: (a) in water - very little change; amount of activity decreased towards shoot tip; very little activity at the tip; no change in gradient, and (b) in solution of cold DNA - change of gradient and thus movement.

These results suggest that the cold DNA is displacing the hot DNA. We do not know what this means, but it suggests an effect of masking surfaces; rather than a genetic or transformation effect it may be a physical effect. (Kuc)

It is easier to cause infection of leaf petioles than leaf blades following injury and artificial inoculation in the greenhouse. (Keil)

Difference in blade and petiole susceptibility appears to be associated with the amount of water in the intercellular spaces. (Billing)

Following two artificial inoculations of 40 cm succulent apple shoots and subsequent separation of plant material into 4 susceptibility classes, there is no evidence for support of the idea for induced resistance. (Cummins)

CONTROL MEASURES

Chemical Control of Fire Blight in Pear by Means of Tree Trunk Injection

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Pome fruit trees usually are protected from fire blight infection by periodic sprays of protective chemicals such as copper or antibiotic. However, once the causal bacteria gain entrance into the tree, there is little one can do to eradicate the disease. This is especially true of certain pear cultivars such as Magness and Seckle where infection tends to concentrate as cankers in the trunk and base of large scaffold limbs. When this happens, one can almost be certain that the tree will die before the end of another growing season. In fact, every one of about forty trees observed with trunk cankers died in the same year that infection was detected.

Preliminary studies at Beltsville indicate that pear trees with trunk cankers respond to chemical treatment by means of trunk injection. The best treatment in these tests was a new experimental organic chemical (MBR-10995) synthesized by the 3M Company. This is the same compound which protects apple and pear trees from blight infection at least equally as well as streptomycin when applied in periodic foliar sprays.

Reported here are results obtained in Magness pear trees in their eleventh leaf after transplanting to the field. The trees were about 15-20 feet tall with a limb spread of about 15 feet. Trunk diameters measured 8-9 inches at one foot above ground level. These trees showed large trunk cankers extending 2-4 feet into the base of some of the scaffolds. At the time of treatment, cankers appeared dark with or without oozing. There was no evidence of shoot blight in the test trees at any time during the study.

Materials and Methods

Tree number 1 had normal size leaves on 3/4 of the tree and leaves 1/2 - 3/4 normal size on the remainder with a medium fruit crop on most of the tree. All of the foliage appeared light green compared to dark green foliage in healthy trees. One dead branch on the southeast side was 3 inches in diameter at attachment to the trunk. Trunk canker without oozing extended for about 3 feet into the main leader and into the base of another lateral branch. Treatment was applied May 13, 1974.

Tree number 2 had normal size leaves with a medium fruit crop on half of the tree and leaves 1/2 normal size with relatively few fruit on the remainder of the tree. All foliage appeared light green. A large oozing canker was present in the trunk which extended into the base of three large limbs. Treatment was applied June 7, 1974.

Tree number 3 had normal size leaves on 1/4 of the tree. The remainder of the tree showed leaves 1/4 to 1/2 normal size with some wilted leaves indicating insufficient water to maintain turgor pressure. One large limb was dead on the south side of the tree. A trunk canker extended into the base of all scaffold limbs. Internal bark at ground level appeared brown when examined by cutting into it with a knife. Treatment was applied May 13, 1974.

Tree numbers 4, 5, and 6 were similar to those mentioned above but no treatment applied. These trees were considered as checks and were all dead before the spring of 1975.

Trees number 1, 2, and 3 were gravity fed with 28 ml (10 grams active ingredient) of MBR-10995-3S in 1000 ml distilled water in each of 3 sites per tree. Holes 2.5 inches deep, 1/4 inch diameter were drilled in the tree

trunk. The three holes were arranged in a spiral around the trunk so that any two holes would not be in the same plane. A female plastic quick-connector was gently tapped into the hole with a hammer until the flange was flush with the bark. A male plastic connector attached to a hose leading to a vessel containing the test solution was attached to the female connector after the solution started to flow. Usually the test solution was taken up by the tree within 1.5-2 hours.

Results

In May 1976, two years following injection, 2 of the injected trees No. 1 and No. 2) were still living. All the check trees died as well as Tree Number 3 which was extensively infected before injection with MBR-10995-35. Both of the living trees appeared to be making a slow recovery from the serious infection they had contracted 2 years before. It is suspected that recovery would have been more rapid if the injection treatment had been made before the damage became so extensive. Because these results were so unusual and spectacular, one may speculate that this system of control might be used as a prophylactic treatment before infection occurs and as a substitute for foliar spray applications.

Biological Control of Fireblight
with Bacteria Antagonistic to *Erwinia amylovora*

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Control of fireblight in California is currently based upon the deposition of bactericides in flowers. Since infections of pome fruits principally originate in flowers, an analysis of the disease cycle suggested that it might be possible to effect a biological control since unopened flowers are sterile and can subsequently be colonized by saprophytic bacteria which are inhibitory to *Erwinia amylovora in vitro*. Saprophytic bacteria were selected for their ability to inhibit the growth of *Erwinia amylovora in vitro* and to multiply in pear flowers. Ultimately, four different bacteria were tested on one orchard in Winters and one orchard in Sacramento, California with four replications per treatment and four trees per replication. Treatments were made weekly and corresponded with applications of fixed copper. Applications did not begin until *E. amylovora* was detected in a mass sample of flowers using our monitoring procedures.

The fixed copper sprays were very effective and controlled 95% and 97% of the disease in the Winters and Sacramento orchards respectively. Antagonists 3a and 14 controlled 55% and 53% of the disease respectively in the Winters orchard when compared to the check plots. (Table 1) However, antagonists 3a and 14 only controlled 1 and 11% of the disease respectively in the Sacramento orchard. These results indicate that biological control of fireblight with antagonistic bacteria may be possible under some conditions.

However, a successful biological control program will be dependent upon finding an organism that can successfully compete with *E. amylovora* under all climatic conditions. In addition, the degree of control must compare economically and efficaciously with that of present chemicals or it will not be accepted by growers.

Table 1

Fireblight Control Tests On Pear Flowers Sprayed
With Fixed Copper Sprays or Pre-Inoculated with Antagonistic Bacteria

Winters		
Treatment	Strikes/tree	% Control
fixed copper ^x	1.1 a ^z	95
antagonist 3a ^y	10.3 ab	55
antagonist 14	10.8 ab	53
antagonist 21b	14.1 bc	39
antagonist 3c	17.6 bc	23
check	23.0 c	
Sacramento		
fixed copper ^x	.8 a	97
antagonist 21b	20.8 b	37
antagonist 3c	27.6 bc	16
antagonist 14	29.1 bc	11
antagonist 3a	32.5 c	1
check	32.8 c	

x = six applications of Kocide at 56 Kg/ha approximately every 4 days beginning April 2.

y = applications of antagonistic bacteria (10^7 cells/ml) sprayed to run-off on the same days as application of Kocide.

z = values followed by different letters are significantly different at the 5% level as determined by Duncan's multiple range test

Fire Blight Control in California in the 1970's

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At the beginning of this decade it was common springtime practice in California Bartlett pear districts to begin regular 3-5 day spray and dust treatments at 5% bloom, and to continue for 2 months or more. This heavy reliance on chemical control of blossom blight has been necessary since mild winter temperatures encourage prolonged pear bloom and flowers continue to appear well into the growing season, but it could mean as many as 15-20 treatments. Streptomycin and copper formulations were the predominant bactericides, although the former was favored because of its lack of phytotoxicity and subsequent enhancement of fruit quality. Copper materials, even at low concentrations, damage the lenticels of young fruit.

Following a major epidemic in 1970 and surveys which suggested that effective blossom blight control had been achieved by frequent applications of relatively low rates of streptomycin (60-240 ppm), use of streptomycin was intensified in California. Not surprisingly, a serious problem became apparent during the 1971 season; severe blight developed in Sacramento Valley orchards where growers had followed a thorough streptomycin program. The predominant strains were highly resistant to streptomycin and continued to be so in succeeding seasons despite a reversion to copper treatments; this added a new dimension to blight control studies and underlined the need for an integrated approach to control.

Until E. amylovora monitoring techniques were developed, a void remained in information relating inoculum levels of the pathogen to temperature regimes and infection. Once it had been established that in most years under California conditions the pathogen first appeared in healthy blossoms as an epiphyte prior to disease development, the way was open to successful prediction plus more timely and economic use of the bactericides.

Thorough field testing and refinement of the selective medium over the past few years have demonstrated its value in monitoring fire blight potential. Pest management consultants have availed themselves of the new technique, and many growers, using monitored data as they became available each season, have altered their blight control programs, frequently to considerable economic advantage. Some growers have been using 5-6 treatments in place of the previous 15-20! Conservatively extrapolating across the 40,000 acres of bearing pears in California, it is estimated that the average grower has been able to eliminate 3 treatments in each of the last 2 years for a total state savings approximating \$500,000/season.

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CONTROL OF SHOOT BLIGHT OF PEAR USING STREPTOMYCIN

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Fire blight is a problem that has beset Ontario growers for many years. Occasionally the disease reaches epiphytotic levels. The losses are not only short term as in the case of fruiting wood but long term when the disease runs down into the scaffold limbs and the trunk of the tree.

Growers have managed to escape disease by maintaining their trees in a state of low nutrition through a program of reduced fertilization, however they have learned to live with the resulting low yields that such trees provide. The alternative system would be to fertilize the trees to provide good returns and undertake disease control measures for fire blight. One such measure is the use of streptomycin.

The spray calendar recommendation for fire blight control in Ontario is based on that adopted for fruit trees in New York and Michigan where blossom blight is much more prevalent. However, in this area, blossom blight is rarely seen and twig blight is the predominant form of fire blight. The limited data on the efficacy of streptomycin for control of twig blight warranted further research into this problem.

Plots sprayed with 100 ppm streptomycin following periods of windy, wet and warm weather from bloom through 30 days before harvest and unsprayed check plots were assessed for blossom and twig blight throughout the summer months in a commercial pear orchard.

Blossom blight was not observed in any of the three years in which the investigation was conducted. Twig blight, on the other hand, was present and the data indicated that significant control was achieved using streptomycin on an extended spray schedule (Table 1). Cost of this control is quite expensive but with elimination of blossom sprays because of the absence of blossom blight the cost would be more reasonable for the grower wishing to increase pear production without increasing fire blight incidence.

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TABLE 1. EFFECT OF FOLIAR APPLICATION OF STREPTOMYCIN
ON FIRE BLIGHT SEVERITY IN PEAR TREES

Treatment	Strikes ^a		
	1974	1975	1976
Control (not sprayed)	600	822	114
Streptomycin (100 ppm)	111** ^b	38**	8**

^aValues are based on four replicates of five trees each.

^bAsterisks ** indicate that streptomycin treatments are different from their respective controls at $P = 0.01$.

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An experiment was conducted at the Agway Farm Research Center at Fabius, New York, to evaluate the value of trunk injection with Tetracycline in curing apple trees infected with fire blight. Apple trees of the cultivars 'Spartan' and 'McIntosh' had been infected with the disease as a result of artificial inoculation during bloom with Erwinia amylovora.

On July 1, 1976, four trees were injected with 1, 2 or 3 ml of a slurry containing either 0.5 g/ml of Tetracycline or 0.5 g of Tetracycline plus 0.25 g of Benlate 50W/ml. Injections were made by drilling one or two 1/4" and 1 1/2" holes in the tree at 45°, pipetting the chemical slurry, closing the hole with a wooden plug then treating the cut surface with tree wound paint. Visible cankers in infected branches were marked with white paint at the time of injection and the advance of infection was evaluated one month after treatment.

Results indicated that injections with Tetracycline were effective in retarding the advance of fire blight in the infected shoots and provided partial cure of infected trees. Treatment with 1 and 1.5 g/tree were more effective than treatment with 0.5 g/tree.

The progress of shoot infection of fire blight was inhibited by Tetracycline treatments and such inhibition was correlated with the dose of Tetracycline/tree. Slight phytotoxicity symptoms in the form of terminal leaf chlorosis appeared on the treated trees. Such symptoms were more pronounced on the 1 g and 1.5 g/tree treatments and on branches with heavy fruit load; however, most of the phytotoxicity symptoms disappeared later in the season.

Table 1. Effect of injection with Tetracycline on fire blight infection on apple trees. (1)

Injection Treatment and Rate in mg/tree	Phyto. Index (2)	Infection progress in cm		Curative Index (4)
		Spur (3)	Terminal	
Check (No Injection)	0	8.7 a	13.5 a	0
Tetracycline 500	1	1.3 b	6.8 b	3
Tetracycline 1000	2	0 c	5.3 c	4
Tetracycline 1500	3.5	0 c	4.2 c	5
<hr/>				
Check (Benlate Alone)	0	9.3 a	16.9 a	0
Tetracycline 500 + B	0.5	2.1 b	4.5 b	2
Tetracycline 1000 + B	1.3	1.0 c	4.1 b	4
Tetracycline 1500 + B	2.5	0 d	3.8 b	5

(1) Mean of 8 infections/treatment.

(2) Phytotoxicity was evaluated based on 0-5 index, where 0 = no symptoms and 5 = severe leaf chlorosis.

(3) a. Infection progress in the term of the length of canker advancement as a result of either spur or terminal shoot infection since injection time.
b. Means within each column and within each group with the same significant letter are not significantly different at the 5% level of Duncan's Multiple Range Test.

(4) Curative values are based on an index of 0-5, where 0 = no cure and 5 = complete and speedy recovery from fire blight advancement in the shoot.

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Glyodin Solution (30% 2-heptadecyl-2-imidazoline acetate in 2-propanol) is a protective fungicide registered for the control of apple and pear scab, "summer diseases" of apple and several other tree-fruit diseases. The preparation has good surfactant properties. Earlier work in Missouri indicated that it is compatible with streptomycin and increases streptomycin absorption on treated trees.

Agri-Strep^(R) was evaluated at two concentrations with and without Glyodin Solution in the Agway Research Orchard near Fabius, NY. Each material was applied to a block of four 6-year-old Malus pumila trees (two cv. McIntosh, two cv. Spartan) in different tree rows. Materials were applied to run-off with a handgun at 400 psi at 50% bloom (10 May 1976) and at full bloom (12 May 1976).

To encourage development of disease, blossom clusters were inoculated with a potassium phosphate buffered suspension of Erwinia amylovora containing 1×10^7 viable cells/ml on 11 May 1976 during a rainstorm of moderate intensity. Erwinia amylovora had been grown in liquid shake culture prior to freezing in pasteurized non-fat dry milk. Inoculum was applied with a cone-jet tipped wand to runoff at 30-40 psi.

All blossom or fruit clusters were examined one month after inoculation to determine the percent infected by fire blight. The results are presented in Table 1. There were no significant differences in the control of fire blight with streptomycin applied at the higher (100 ppm) rate with or without Glyodin Solution or with the lower rate with Glyodin Solution. However, control was significantly poorer at the lower (50 ppm) rate of streptomycin alone. When Glyodin Solution was added to Agri-Strep at the lower rate, control improved to the same level achieved with the higher rate. Use of Glyodin Solution with Agri-Strep during bloom should be considered because of its effectiveness as an adjuvant with streptomycin and its fungicidal properties.

Table 1. Control of fire blight blossom infection

Material	Rate/100 gallons	Percent Clusters Infected ¹	Percent Disease Control
Agri-Strep	0.5 pound (100 ppm)	0.6 a	97.6
Agri-Strep plus Glyodin Sol'n	0.5 pound (100 ppm) 1 pint	1.3 a	95.0
Agri-Strep	0.25 pound (50 ppm)	7.1 b	73.4
Agri-Strep plus Glyodin Sol'n	0.25 pound (50 ppm) 1 pint	1.8 a	93.4
Water	---	26.5 c	---

¹Percent infected cluster data followed by the same letter do not differ significantly ($P = 0.01$).

RECENT RESEARCH ON AND CURRENT
RECOMMENDATIONS FOR FIRE BLIGHT CONTROL IN NEW YORK

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Control of fire blight is difficult in New York because of the sporadic nature of the disease. Growers are often hesitant to implement costly control recommendations because they have little assurance that the disease will cause severe losses in any given orchard or season. The same uncertainty of disease occurrence and severity that plagues the grower, also affects the scientist who endeavors to develop and test improved control measures. The success rate of test plots in commercial orchards has been disappointing; approximately five out of six plots fail for lack of sufficient disease development to permit evaluation of test treatments.

As a consequence of a severe fire blight epiphytotic on apple in Western New York in 1972, increased research efforts on fire blight control were supported by the College administration. To improve the efficiency of control testing, a young apple orchard was leased from a private grower in Wayne county for the sole purpose of conducting research on fire blight epidemiology and control. The lease provided for routine maintenance of the orchard by the grower with the understanding that trees were likely to be killed or rendered permanently commercially unproductive. Techniques for the reliable production of blossom infection were developed, involving artificial inoculation with Erwinia amylovora, that ensure the development of sufficient infection to evaluate control measures [Phytopathology 64:478 (1974) and Fungicide and Nematicide Techniques (Submitted, August, 1976)].

Spray materials that had been recommended in New York, those recommended in other areas, and experimental compounds have been evaluated during the past four growing seasons. Tests designed to indicate the best scheduling and methods of application of effective spray materials were carried out to improve recommendations to growers. The result of these tests are summarized briefly below.

1. Properly timed streptomycin sprays are very effective in reducing the occurrence of blossom blight.
2. Use of adjuvants (glycerin, Regulaid, or Glyodin Solution) in combination with the Agri-Strep formulation of streptomycin increases the efficacy of the antibiotic [Fungicide and Nematicide Tests - Results of 1974, 30:10 (1975); Plant Dis. Rep. 60:541 (1976)].
3. The performance of "fixed" and "liquid" coppers on apple in New York has been disappointing (relative to streptomycin) based on both efficacy and phytotoxicity [Fungicide and Nematicide Tests - Results of 1973, 29:6 (1974); Fungicide and Nematicide Tests - Results of 1975, 31:14 (1976)].
4. Streptomycin was less effective when applied in 25 gallons of water per acre than when the same amount was applied in greater water volumes. Addition of an adjuvant increased apparent degree of control at the 25 gallons per acre rate [Plant Dis. Rep. 60:541 (1976)].

5. The most promising experimental material evaluated was produced by the 3-M Company (MBR-10995). It compared favorably with streptomycin [Fungicide and Nematicide Tests - Results of 1974. 30:10 (1975)].

An integrated program of fire blight control is recommended in New York that is based on both past and recent research. ["Fire blight - its nature and control", (N.Y.S. Coll. Agri. and Life Sci., IB #100, 16p., 1976)]. Current spray recommendations are discussed in the College's annual "Tree - Fruit Production Recommendations for Commercial Growers".

Control recommendations are aimed at reducing disease severity by three means, reduction of inoculum, reduction of host susceptibility and preventing host and pathogen from interacting.

Inoculum reduction is to be achieved by removal of overwintering cankers from the orchard and its vicinity, removal of early-season blossom infection ("patrolling") and by early-season sprays of Bordeaux mixture plus oil.

Reduction of host susceptibility is to be achieved by selection of orchard sites not conducive to severe blight development (well-drained, non-acid soils), selection of scion and rootstock cultivars that are less-susceptible to severe fire blight damage, soil management practices (lime and nutrient application) that result in the growth of less-susceptible host trees, and pruning practices that remove unnecessary tree structures (trunk spurs, root and water sprouts) that if infected may lead to severe tree damage.

The interaction of pathogen and host is prevented directly by application of materials that inhibit the pathogen and indirectly by control of insect vectors.

Pertinent Statements Made During Discussion on Control

Several organisms antagonistic to Erwinia amylovora have been isolated from orchard soils. They have been reisolated from flowers sprayed with these organisms and they spread from sprayed to unsprayed flowers. (Thomson)

Based on observations of blight development in Ontario, rattail blooms do not seem to be more susceptible than shoots. (Bonn)

A new experimental organic compound developed by the Dupont Company shows promise in control of E. amylovora, E. cavatovora and Xanthomonas vesicatoria. (Davidson)

"Teneyck" control of fire blight in Wisconsin consists of periodic burning out of any visible blight with a propane torch. (Wade)

When E. amylovora is not monitored, it is probably advisable to apply streptomycin sprays during the bloom period and possibly just before bloom in order to build up antibiotic titre in the plants to keep down the number of E. amylovora bacteria. (Goodman)

Streptomycin-resistant E. amylovora was originally (early 1970's) observed in 5-6 orchards in California; today it is known in 5 counties of this state. (Moller)

Because oxytetracycline (terramycin) has shown considerable promise in controlling strep-resistant E. amylovora in California, attempt is being made to have this material registered for use only in the western states by spring 1977. (Carroll)

Terramycin has to be used at much higher concentrations than streptomycin to give comparable control of strep-susceptible E. amylovora under Maryland conditions; therefore, it would not be used even if it was registered. (Keil)

Control of rattail bloom is extremely important in the overall control of fire blight. If it could be prevented following normal fruit set, fire blight control could be simplified. (Beer, Moller)

FIRE BLIGHT

IN EUROPE

Current Status and Epidemiology of Fireblight in Poland,
Denmark and Germany by W. Zeller

After the first occurrence of fireblight (*Erwinia amylovora*) in Europe in the United Kingdom in 1957 until now the disease spread to the following countries: Netherlands (1966), Poland (1966), Denmark (1968), Fed. Rep. of Germany (1971), Belgium (1972), France (1972), German Democratic Republic (?). The bacteriosis as yet is limited to the northern regions of the continent but it seems to spread further to the south. A survey of the current status of distribution in Poland, Denmark und Germany shall be given here.

Poland: A detailed account of the fireblight situation in Poland was given by BURKOWICZ in 1972. According to him the first outbreak of fireblight was detected in the summer of 1966, in the experimental orchard of the Research Institute of Pomology at Milibadz, about 16 miles south of the main sea port Gdansk. Initially symptoms were only found in one block of 8 year old pears with 22 varieties. By the end of September, over 50 % of the trees had been completely destroyed. The varieties most affected were Bartlett, Conference, Covert, Princesse Marianne, Countess de Paris and Beurre Bosc. At the end of the summer, fireblight was also observed on eight - year - old apple trees growing near the infected pears. Some trees were completely destroyed.

According to BURKOWICZ the reasons for the extremely severe fireblight outbreak were as follows:

Severely pruning measures because of frost damage, heavily sterilizing, unusually warm summer combined with the high humidity characteristic for this area. ^{fertilize}

In the summer of 1968 a mild case of fireblight was observed on a single pear tree in a pear collection at SKIERNIEWICE (Central Poland). The tree had been propagated in the spring of 1968 with grafts of the cultivar Conference, obtained from the United Kingdom.

A further outbreak of the disease was discovered at the end of September on young old apple-trees in a nursery at Dworek, 4 km from the seashore. The disease symptoms were limited to a few apple-trees and some old hawthorn bushes in a hedge. The last report of fireblight has been in September 1971 from two pear orchards, located 40 km south of Gdansk. Only 7 trees were attacked.

Since that time no further outbreak of the disease could be discovered probably as a result of intensive eradication procedures. All pear blocks and apple-tree blocks containing even a single infected tree, the hawthorn hedges and the entire nursery were uprooted and burnt. All wild pear and apple-trees, hawthorn and mountain ashes growing within a half mile radius of the infected orchards were also grubbed up and burnt. For example in 1966 more than 5000 trees were destroyed. According to SOBISZEWSKI from the Research Institute of Pomology in Skierniewice nowadays every year were carried off two inspections for fireblight control in pear and apple orchards: one in the spring and second in autumn. A special attention is put out on the north of Poland and seacoast, but fireblight was not discovered.

Denmark: The first outbreak of fireblight was detected in late August 1968 on the northern portion of the Baltic Sea island of Falster. The disease was found on several pear and apple varieties, a single bush of Cotoneaster watereri and many hawthorn. Despite of intensive eradication procedure in this area fireblight recurred in 1969 after the beginning of August in 80 different locations on the northern portions of the island of Falster and Lolland, as well as on the smaller islands of Femø, Fejø and Askø, located between the two larger islands. At the end of the year 1969 in 288 locations predominantly in hawthorn hedges surrounding the orchards fireblight could be found.

In the next year 1970 a small attack was found on the island Langeland and severe infections in an orchard on Sealand. According to JACOBSEN in early August 1971 the situation in Denmark totally changed. At that time severe and widespread attacks were found in the western part of South Jutland right north of the Danish-German frontier. The area is typical agricultural country without orchards, so the infections were nearly solely on hawthorn, in these areas extensively grown as wind-breaks around farms and fields.

These discoveries had the effect that the Danish Ministry of Agriculture decided that it could no longer pay the expenses of the eradication program and so the destruction of infected host plants was immediately stopped.

Since that time the disease could be considered as established in Denmark and the destruction of infected host plants was ordered or only recommended where special economic interests were involved, for example fruit growing and nursery production.

With the exception of 1972, where on the island Funen a new focus was detected, until 1975, no real spread of fireblight was found. A further spreading took place in 1975. According to JØRGENSEN the infected area in the southwestern part of Jutland has moved about 10 km towards the east.

A new infection area has been established in the southeastern part of Jutland (east of the town Haderslev); a single occurrence of the disease was found near Odense (the island of Funen) and a few findings in the Copenhagen area, both west and north of the town.

According to the 24th Annual Report of the Danish Government Plant Protection Service for the year 1975 the geographical distribution of new cases of fireblight was as follows:

Western Jutland	3	localities
Southern "	39	"
Funen	10	"
Zealand	20	"

These findings were made on the following host plants:

Cotoneaster cornubia, *C. salicifolius floccosus*, *C. watereri* "Brandkjaer", *Crataegus monogyna* and *oxyacantha*, *Pyracantha coccinea*, *Pyrus malus* and *Stranvaesia davidiana*.

JØRGENSEN means that it is remarkable that until now hawthorn appeared as the main host plant, but from now on also *Cotoneaster* species seem to become important host plants. Especially *C. salicifolius floccosus* and *C. watereri* have been dominating in the new findings on Zealand.

Fed. Rep. of Germany: Nearly at the same time in 1971, when fireblight has been occurred in Jutland the first outbreak was detected in the northern part of Schleswig-Holstein between the North-Sea island Sylt and the peninsula Eiderstedt. The disease appeared on more than 40 locations especially on hawthorn. Drastic eradication measures were immediately undertaken. According to MEYER from Plant Protection Service nearly 11000 plants, mostly hawthorn bushes were removed from the infected area and burned.

Despite of this extensive eradication campaign the disease occurred in the next year 1972 and spread to the south and east, so that new fireblight locations could be found in the following, federal country of Niedersachsen at the north-eastern sea coast. In order to prevent the spread of the disease further 19000 plants have been destroyed. During 1973 and 1974 only a weak course of infection happened perhaps because of dry spring and summer in these years. Since that time in the contaminated zone of Schleswig-Holstein a general destruction of infected host plants could not be carried out, because the government could not pay longer the high expenses. So only around nurseries host plants, mostly hawthorn hedges in a radius of 3 km, were eradicated to prevent the possibility of spreading.

As like as in Denmark a further spread of the disease took place in 1975. The bacteriosis moved from the sea coast of

Niedersachsen about 100 km towards the west to the area of Oldenburg, where first cases of fireblight were found in parks and nurseries. Further findings were made in the area of the sea-ports Hamburg and Bremen.

The attacked host-plants in 1975 were mainly Cotoneaster with the variety C. salicifolius floccosus.

German Democratic Republic: A single occurrence of fireblight was found in the north of the Republic near the Baltic Sea coast. The focus was reportedly totally eradicated.

EPIDEMIOLOGY AND CURRENT STATUS OF FIRE BLIGHT
 IN THE NETHERLANDS, BELGIUM AND FRANCE (25)

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The Netherlands

The epidemiology and the current status of fire blight in the Netherlands up to the summer of 1974 have been previously described (lit. 1). Up to that time the situation appeared to be under control thanks to eradication actions. Infection was limited to two relatively small foci, one in the south western part of the country and the other in the north western part. It was mainly found in hawthorns, much less in *Cotoneaster* (mostly in *C. salicifolia* and *C. watereri* and their cultivars) and only sporadically in *Sorbus* (*aria*) and pears (*Pyrus communis*).

In the second half of September that year, however, three new foci were discovered in different parts of the country. All three new foci, which had no apparent connection with the two old ones, were distinctly *Cotoneaster*-foci. How the inoculum arrived in these new foci is unclear. Most likely several infected bushes had been present in each of these foci which produced inoculum that was later spread by the summerstorms of July and August 1974. Storms in that time of the year occur seldom.

Surveying and eradication actions were carried out during 1974 and the first half of 1975. Infection then appeared to be restricted to the five foci known at the end of 1974. However, by the end of July 1975 this picture began to change. During August that year it became quite clear that fire blight had spread over other parts of the country, although in several areas still no infection was found. Infection was found to occur mainly in *Cotoneaster* (mostly via blossom infection), much less in hawthorns and only incidentally in *Stranvaesia*, *Pyracantha*, pears (*Pyrus communis*) and *Sorbus*.

Nearly all infected and suspected bushes and trees were destroyed under legal enforcement. Infections were also found in a number of nurseries. In these cases all plants of the infected species or cultivar were destroyed.

The situation in 1976 is so far not very encouraging. Outside nurseries many infected bushes have been found, spread over the country, but these appear mainly to be restricted to the two very susceptible and sensitive *Cotoneaster* species *salicifolia* and *watereri*. Also in several nurseries some infection has been found although much less than in 1975. Again all plants of the infected species or cultivar grown in these nurseries have been destroyed immediately. Furthermore the culture of the susceptible species mentioned and their cultivars is to be forbidden in the main Dutch nursery-centre as per January 1, 1977.

Planting out of these species in towns and villages is now discouraged. Planting of these species as well as of *C. dammeri*, *Crataegus monogyna* and *Cr. oxyacantha*, *Pyrus communis* and *P. salicifolia*, *Sorbus aria* and *Stranvaesia davidiana* is discouraged in the direct vicinity of vital industries (orchards and nurseries which grow hostplants).

The spraying of streptomycine has so far been forbidden in this country. However, it has recently been made possible to use this compound for special purposes (nuclear stock field, multiplication plots of virusfree material, researchstations and during the current season in those nurseries where an infection has been confirmed in the nursery concerned or in the immediate vicinity).

Belgium and France

Fire blight was first discovered in Belgium in the south western part towards the end of 1972 in hawthorns (lit. 2). This focus extended into the north western part of France, where infection was found previously during 1972. Here too infection occurred only in hawthorns (lit. 3).

In both countries eradication actions were started immediately. In France the eradication was already carried out in 1972 by removing all diseased and many of the other hawthornshrubs. Here the good luck existed that infection was only present in a meadow-region with scattered hawthorns. Since 1972 no infection has been found in France.

Eradication in Belgium was more difficult because of several reasons. The landscape where the action had to be carried out was more complex of structure. Technically it was tried to burn down the diseased hawthorns with the help of flame throwers, which did not succeed. Nevertheless they were successful in almost completely eradicating this infection towards the end of 1973. Only a small spread has taken place in 1976 in this region and in an area close to the Dutch border. Mainly *Crataegus* and *Cotoneaster* have been infected as well as a few pear trees. Eradication actions are being continued. The cultivation of 5 of the most susceptible and sensitive species of *Cotoneaster* has been forbidden in all Belgian nurseries since the first of May 1976.

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Fireblight was first found in England in 1957 in pear orchards and by 1960 it was realised that eradication was impossible. Infected hawthorns were first found in 1959 and in 1960 many ornamental hosts (eg. Sorbus spp., Cotoneaster spp. Pyracanthus spp.) were severely infected as well as pears and hawthorns. In apple orchards, in spite of their proximity to infected pears and hawthorn hedges, no infections were confirmed until 1967. Since 1960 the amount of infection in different hosts in different seasons has fluctuated widely.

In pear orchards, worst infections have been seen on trees carrying summer blossom (including rat tail bloom). Only in one year (1968) has severe spring blossom blight been seen and that was largely confined to a single cultivar in one orchard. Shoot tip infections have rarely been seen and in my own limited experience, only in association with blossom infections. In some years (1957, 1966 and 1967) out of season blossom was common on some pear cultivars and fireblight in pear was severe in those years. One highly susceptible cultivar (Laxton's Superb) commonly produced blossom throughout the growing season and was the pear most commonly infected.

Severe infection on hawthorns has varied widely from season to season. Shoot tip infections are commonly seen alongside blossom infections but rarely during the remainder of the growing season except following storms. Shoot growth occurs prior to bloom on this host and shoot infections sometimes precede blossom infections.

Information on infections on ornamentals tends to be sporadic; there may be many infections in private gardens which go unreported. There is no suggestion that the disease has been as widespread and severe and in such a wide range of hosts in streets, parks and gardens as it was in 1960 (mostly in the southeast suburbs of London and in the Southern area).

Infections in apple orchards have been almost entirely confined to the years 1967 and 1969. In 1967, blossom infection was common in one late flowering cultivar (Crawley Beauty) in a single orchard and sporadic infections were seen in another on an earlier flowering cultivar (Charles Ross). On the former there was good evidence that infection was present on the trees in 1966 (Lelliott, 1968). In another orchard about 80 miles to the north, shoot infection was seen in late August on young trees of cv. Egremont Russett and on mature trees of cv. Miller's Seedling. In the same orchard the following year (1968), there was widespread shoot in the same cultivars and occasional strikes in others following a severe wind/rain storm in early July. The source of infection in both years was undoubtedly infected hawthorns. By coincidence, in 1969, there was again a severe storm in early July and this time apple shoot infections were seen over a wide area of southeast England. In nearly every case there were infected hawthorns in the vicinity and the evidence left little doubt about wind/rain dissemination of inoculum from infected hawthorns to wind damaged apple shoots (Glasscock, 1971; Billing, 1974). Since 1969 infections in apple orchards have rarely been seen.

In England, epidemic blight of fruit blossom in spring is likely to be very rare unless our climate changes or later blooming cultivars become common. Isolated infections however do occur in some years and, if undetected, are a potential source of infection later in the season so we cannot be complacent about the situation in the spring blossom period (about mid-April to mid-May). Surprisingly, infection has been seen more commonly on pear than on apple blossom.

The greatest danger to fruit trees, as in other parts of northern Europe, is from alternate hosts which flower later in the season eg. Crataegus and Sorbus spp. (May to mid-June) Pyracanthus spp. (June), Cotoneaster spp. (June to July). These flowering periods overlap the main summer blossom periods on pear and the periods of most active shoot growth on both pear and apple. No orchard is remote from wild Crataegus spp. and many growers still use them as windbreaks; few are far from gardens with susceptible ornamental shrubs. Even so, the risk to pears may depend largely on the amount of blossom they produce in summer and the risk to apple (which at present seems very low) on unusually severe storms, unless changed cultural practices increase their vulnerability.

Comparison with early experience in Denmark is of special interest. Fruit blossom periods in the affected areas are later than those in England but temperatures are not necessarily higher at the time. There, primary blossom infection in both pear and apple blossom were seen before temperatures above 18°C had been recorded. Hawthorn hedges were undoubtedly the main source of inoculum (Bech-Andersen, 1971, and unpublished data). So far there has been no suggestion that, in Denmark, summer blossom on pear presents a fireblight hazard or that apple shoot infections are a problem.

In England, grubbing and burning all infected trees was the policy until 1968 when cutting out infected parts under supervision was allowed. By 1970, the highly susceptible pear cultivar Laxton's Superb (which was prone to producing summer blossom in most seasons) had been phased out of the main infected areas.

Fireblight is still a notifiable disease but for the fruit grower it is now more of an advisory problem than a statutory one. Since 1970, few special searches have been made, so the present status of the disease in England is uncertain. There is no evidence that it has spread to new areas since 1970 but there is no doubt about its continued presence.

With the help of a predictive system which is being developed (Billing, 1976 and unpublished data), it is possible to re-examine the history of fireblight in England and its relation to that in northern Europe and to make an indirect assessment of the current position.

The data suggests that, since 1970, weather in England has been less favourable for fireblight activity than it was in earlier years and that severe hawthorn blossom infection was unlikely. The most favourable period was between 1964 and 1969 when both hawthorn and pear infections were common and severe. Since 1970 hawthorn infections have been few and mainly slight when previously infected trees have been examined.

In recent years, weather was most favourable between June and September 1974 and there seems little doubt that favourable weather that year was an important reason why there was such severe fireblight, mostly on cotoneasters,

in 1975 in northern Europe. England seems to have escaped lightly in 1975 though two severe outbreaks were reported; heavier and more frequent rainfall in continental Europe may well account for the greater severity of the disease there.

One important question now is whether or not latent infections are present in previously (or newly) affected areas which have shown no sign of fireblight in recent years and whether they may be reactivated in the future.

It is interesting to compare experience in Northern Europe with that in North America. In Europe, ornamental tree and shrub production is an important industry alongside the fruit industry and it is unfortunate that some of the most popular trees and shrubs are fireblight hosts. Added to this, Crataegus spp. are used for windbreaks in orchards and species (Cr. monogyna and Cr. oxycentra) which are extremely common in the wild state are all highly susceptible to fireblight, unlike many North American species.

In pear trees, experience in England is more like that in California than in eastern areas of North America. Summer blossom is by far the most common route of infection whilst shoot infection, common in eastern areas, is very rare. In apple in England however, shoot tip infection has been far more common than blossom infection but has only been seen following storms (absence of shoot infection in pears at these times is probably due to the fact that they mature earlier than those on apple).

The implications are that in both England as in California the pear blossom period is often prolonged and there is a greater risk of frost damage or poor set of fruit because of cool weather after pollination and this (in England at least) appears to encourage summer blossom production from June onwards.

In England severe storms are rare during the period of active apple or pear shoot extension but in eastern areas of North America they are common and the combination of wind and rain and possibly hail are favourable for infection of shoot tips which, without damage, are not readily infected by low levels of inoculum.

Slight alterations in climate in any one area affected by fireblight (eg. severe storms in England) may affect the behaviour believed to be characteristic of the disease in that area and it may be difficult to predict the precise pattern of events likely in an area so far unaffected by fireblight. It should however be possible to gain a broad indication by a study of the prevailing weather during the growing season and by looking for features which are comparable to those in other climatic areas at critical periods, especially at blossom time.

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(Eve Billing)

Approximate range of blossom periods
for Southeast England

Cotoneaster lacteus	
Cotoneaster salicifolia	
Stranvaesia davidiana	
Pyracanthus atalantoides	
Sorbus aria	
Sorbus aucuparia	
	{ Crataegus monogyna Crataegus oxycantha
	Apple — cv Cox
	Pear — cvs Comice, Conference

April

May

June

July

↓
Pertinent Statements Made During Discussion on Fire Blight in Europe

Baterievuur (perevuur) is the new name for fire blight in the Netherlands.
(Meijneke)

Pears and apples in the Netherlands do not usually show blossom blight because of cool weather during bloom (April 1 - May 15). Hawthorn sometimes show blossom infection because of later bloom (April 15 - June 15), whereas blight in Cotoneaster is due mainly to blossom infection (June 15 - July 15).
(Meijneke)

Cotoneaster salicifolia and C. watereri and their cultivars are the most susceptible cotoneasters to fire blight. (Zeller)

Climatic conditions in the Po Valley of Italy, the Rhone Valley of France, and central Switzerland appear to be similar to those found in the pear growing regions of the western United States. Therefore, fire blight may become a serious problem in these regions once the organism is introduced.
(Moller)

CAUSAL ORGANISM

Streptomycin Resistance in *Erwinia Amylovora*

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This report is a synopsis of our investigations on streptomycin resistance in *Erwinia amylovora*. Information from these studies has not been published except for the initial reporting of the presence of naturally occurring streptomycin resistant *E. amylovora* strains in pear orchards (Phytopathology 10:1175, 1972) and some data on the nature and distribution of the strains (Calif. Plant Pathology, No. 7, 1-5, Jan., 1972).

Streptomycin resistant strains infecting pear were found in 1971 in Sacramento Valley in a number of locations (Table 1,2). Subsequent to these findings, resistant strains have been detected in many geographical areas in California, including different hosts such as apple, loquat, and *Pyracantha*. Resistant strains were found in Stockton and Southern California on pears that had never been treated with streptomycin. Other bacteria which colonize pear trees, such as *P. syringae*, also were found to be resistant to streptomycin.

Streptomycin resistance occurs in the field at two levels, 20 ppm and 1000 ppm. On Luria's medium, isolates resistant at 1000 ppm were also resistant at 10,000 ppm. In 1976, a survey of three pear growing counties revealed that approximately 50% of the isolates were resistant. One hundred eighteen isolates were resistant to 1000 ppm and above, whereas 11 were resistant to 20 ppm. All isolates were resistant in the orchard where streptomycin resistance was originally discovered in 1971, but in 1975, 50% of the isolates were resistant following a regime where only copper materials were applied.

Although resistance using the disc bioassay appears to be infinite to concentrations of streptomycin, the incorporation of streptomycin in agar and broth media revealed that increasing concentrations have a toxic effect increasing the generation time. (Fig 1.) When plating cells on agar media, the colony count is markedly reduced with increasing concentrations of streptomycin.

The Delbruck fluctuation test showed that resistant cells were present in the natural population and occurred at two levels, 20 and 1000 ppm+, the same levels found in the field. The mutation rate was about 1.9×10^{-9} and 2.2×10^{-9} for the 20 and 1000+ streptomycin resistant mutants, respectively.

Preliminary tests suggested that streptomycin resistant strains were of greater virulence than susceptible strains. More extensive testing, however, indicated that the variabilities of virulence in susceptible strains was approximately the same as resistant strains (Table 3).

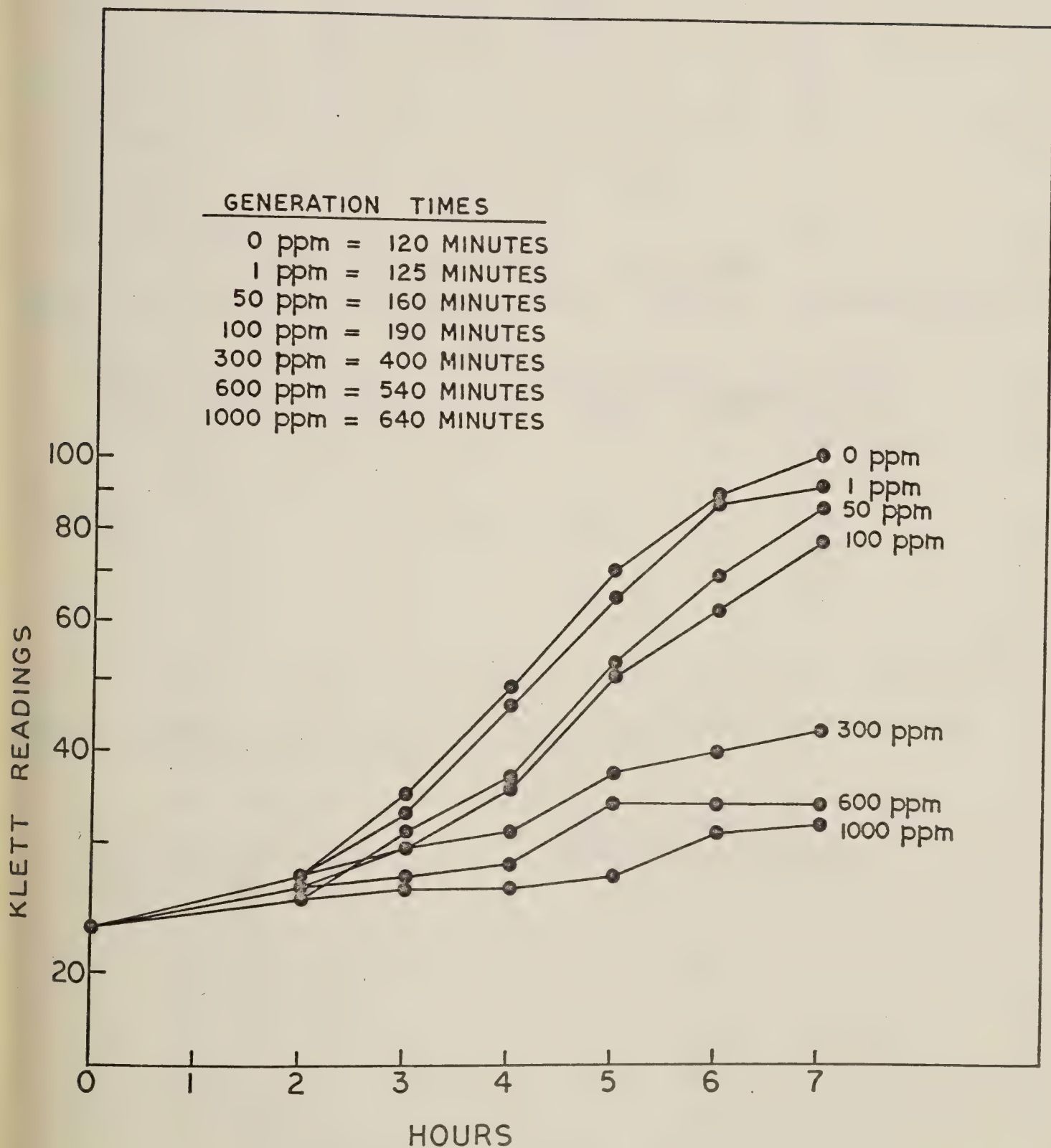


Table 1 : Frequency of Streptomycin Resistance in Different Locations in 1971.

Area	Number of Samples	Number Resistant	Percentage
Hamilton City	18	9	50
Gridley-Live Oak	34	32	96
Yuba City	12	2	18
Marysville	9	4	44
Yolo County	4	0	0
Total			
Sacramento Valley	77	47	61

Table 2: Distribution of Streptomycin Resistant *E. amylovora* in Sacramento Valley, 1971.

Years Using Streptomycin	Total Samples	Streptomycin Resistant Samples	
		Number	Percent
6 - 10	11	6	55
4 - 5	21	14	67
3	3	3	100
2	28	19	58
1	14	5	36

Table 3: Comparison of Symptoms Caused by Inoculating shoot Tips of Pear With Resistant or Sensitive *E. amylovora* Strains.

<i>E. amylovora</i> Strain	Average Distance in cm Blight Ran in Three Weeks
1. Sensitive ^a	0
2. Sensitive	15.5
3. Sensitive	43.5
4. Sensitive	7.5
5. Resistant	43.2
6. Resistant	19.2
7. Resistant	25.0
8. Resistant	27.0

^a This isolate was of low virulence and produces symptoms only when very succulent tissue is inoculated.

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Conjugal gene transfer system, and mapping of genes for plant virulence,
in Erwinia amylovora

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15 September 1976

Paper presented at the Third Workshop on Fire Blight Research,
Ithaca, New York, 20-22 September 1976. A privileged communication.
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Two gene transfer mechanisms, conjugation and transduction, have been extensively exploited to elucidate the physiology of enterobacteria such as Escherichia coli and Salmonella typhimurium. Indeed, it can be stated without reservation that in the absence of these techniques of gene manipulation our knowledge pertaining to the biology of these bacteria would have been sketchy and ambiguous. It is rather unfortunate that knowledge as extensive as that for these enterobacteria does not exist for other bacterial pathogens, including those which cause disease of plants. Bacterial pathogenesis of either plants or animals is a complex process, and is very poorly understood at this time. We feel that a significant contribution to the understanding of the pathogenic process in bacterial phytopathogens necessitates the availability of systems to manipulate the genetic material. We have developed conjugal gene transfer systems in E. amylovora (Chatterjee and Starr, 1973; Pugashetti and Starr, 1975; Pugashetti, Chatterjee, and Starr, in preparation) which are briefly described below.

In addition, we have determined the ability of Erwinia amylovora and other Erwinia spp. to inherit some of the enterobacterial plasmids, and the effects of these plasmids on the virulence of E. amylovora.

Inheritance of enterobacterial plasmids by E. amylovora. The results presented in Table 1 demonstrate that plasmids of various compatibility groups are transferred from Escherichia coli to Erwinia amylovora. The frequency of such transfer in a three-hour cross is relatively high for RP1, and relatively low for F' factors, derepressed R factors R100 and R64, and the human clinical Erwinia plasmid E-lac⁺. We have determined that the sex factor activity--as judged by M13 sensitivity (in the cases of F' factors and R100drd56) and donor ability--of all these plasmids is normal in E. amylovora (Chatterjee and Starr, 1972a, 1972b).

The plasmid-bearing strains of E. amylovora EA178 have been tested for virulence on immature pear fruitlets. We have thus far been unable to detect any effect of these specific plasmids on virulence. It is interesting to note that Bennett and Billing (1976) reported a loss of virulence in strains of E. amylovora resistant to streptomycin. We have not observed any change in the virulence of spontaneous one-step (high level) streptomycin-resistant strains of E. amylovora. It is possible that Str^R strains of E. amylovora--such as those obtained by serial subculture in the presence

of increasing concentrations of streptomycin by Bennett and Billing (1975)-- have multiple genetic lesions. The low level of resistance might have resulted from an alteration in permeability, and the subsequent high level of resistance from an alteration in ribosomal proteins. In the light of our findings on the virulence of mutant strains of E. amylovora with altered barrier property of the cell envelope (Chatterjee, Buss, and Starr, in preparation), it seems possible that an altered permeability of the Str^R strains might have contributed to a loss of virulence.

Isolation of Hfr strains. We have constructed Hfr strains, starting with E. amylovora strains harboring $\text{F}'\text{lac}^+$ and $\text{F}'\text{his}^+$ from E. coli (Chatterjee and Starr, 1973; Pugashetti, Chatterjee, and Starr, in preparation). The procedure followed in each case was the same and, for the sake of clarity, we will describe below (and in Figure 1) the steps followed in the isolation of only one of these Hfr strains (strain 99).

The results given in Table 2 demonstrate that $\text{F}'\text{lac}^+$ is transferred from Erwinia amylovora to E. amylovora, Erwinia chrysanthemi, Erwinia herbicola, Escherichia coli, and Shigella dysenteriae; the frequency of transfer is higher with E. coli and S. dysenteriae recipients relative to Erwinia recipients. It is noteworthy that the frequency of the transfer of $\text{F}'\text{lac}^+$ from E. amylovora to E. amylovora is considerably higher (by about a factor of 10^4) as compared to transfer of the F' factor from E. coli to E. amylovora. We believe this does not result from Erwinia-specific modification of $\text{F}'\text{lac}^+$, since the frequency of the transfer of $\text{F}'\text{lac}^+$ from E. amylovora to E. coli and other erwinias is comparable to the frequency of transfer from E. coli to these recipients. It is likely that this relatively high frequency of $\text{F}'\text{lac}^+$ transfer results from surface properties of these bacteria which contribute to the effective pair formation in homologous matings (Chatterjee and Starr, in preparation).

The $\text{F}'\text{lac}^+$ factor is fairly stable in E. amylovora; the spontaneous loss of the F' factor amounted to about 0.75 per cent in an overnight culture in Penassay broth [Table 3]. However, acridine orange (1 mg/ml) in Penassay broth was quite effective in "curing" the F' factor from these cells; about 90 per cent of cells grown in presence of the dye were Lac^- . The Lac^- clones were also F^- as judged by M13 insensitivity. The stability of the F' factor in the E. amylovora host, the high efficiency of curing of the F' factor, as well as the high frequency of transfer of the F' factor in

homologous crosses all prompted us to look for Hfr strains in the population of F' cells. The procedure adopted is shown as a flow diagram in Fig. 1. We have isolated several Hfr strains using this procedure, and some of their properties are described below.

The data in Table 4 reveal the sensitivity to "curing" by acridine orange of the F' factor, and insensitivity of the Hfr strain 99 to such a curing effect. This is consistent with the finding of Hirota (1960) that acridine dye is effective in curing the F' factor in an extrachromosomal state but not in an integrated state. We have also determined that all Hfr strains of E. amylovora strains that we have constructed using F'lac⁺ and F'his⁺ are insensitive to curing by acridine orange. In addition, we have now obtained molecular evidence of the integration of the F' factor in Hfr strains of E. amylovora. In alkaline sucrose gradients, the lysate of E. amylovora strains harboring F'lac⁺ or F'his⁺ show a peak of satellite DNA, but no such satellite DNA is seen in Hfr strains or in the wild type strain (Pugashetti, Chatterjee, and Starr, in preparation).

Properties of the Hfr strains. The results of the times of entry of several genetic markers from Hfr strains 99 (isolated from an F'lac⁺ strain) and 159 (isolated from an F'his⁺ strain) to E. amylovora recipient strain BP 2158 are given in Table 5. It is evident that, whereas Hfr 99 transfers ser as the proximal marker followed by rbs and ilv, Hfr 159 transfers these markers in a reverse fashion, ilv being the proximal marker, followed by rbs and ser. This behavior suggests, then, that the origins of the transfer in these two Hfr strains are different, as shown in Table 5. We have summarized the frequency of the transfer of various markers from Hfr 99 and Hfr 159 to different recipient strains of E. amylovora [Table 6]. It is also clearly shown that markers which are proximal are transferred at relatively higher frequencies than the markers which are distal--indicating the polarized transfer of chromosomal markers.

We have now initiated a systematic mapping of the E. amylovora chromosome, including the genes involved in virulence. Our analysis thus far has revealed a linkage between ilv and rbs, and between thr and leu. It is noteworthy that in E. coli these markers are located within one-minute intervals of each other. This behavior suggests to us, in a preliminary way, that the gross linkage map of E. amylovora will be similar to those of E. coli and S. typhimurium.

We have analyzed the virulence of recombinants of a cross between Hfr 99 (prototrophic, Str^S , virulent) and BP 2157 (ser thr ilv his rbs Str^R , avirulent) and the results are summarized in Table 7. It is clear that the gene(s) for virulence is (are) linked to ser. The per cent coinheritance for this marker (virulence) with ser is 55 and with rbs it is 25. This places the virulence gene(s) between rbs and ser; it is (they are) closer to ser than to rbs. These results also rule out the possibility that avirulence in this particular strain results from the auxotrophic requirements; ser⁺ thr⁻ ilv⁻ his⁻ mutant strains are both virulent and avirulent; similarly, rbs⁺ ser⁺ his⁻ thr⁻ ilv⁻ mutant strains are both virulent and avirulent (Pugashetti, Chatterjee, and Starr, in preparation). Of course, we do not yet know the gene-product(s) involving virulence of these particular cases. This constitutes a subject matter for our further studies (Chatterjee and Starr, in preparation).

The results presented above clearly indicate that the conjugational genetic systems that we have described are adequate for mapping the chromosomal loci, including the genes for virulence in Erwinia amylovora. We continue to search for suitable transductional systems.

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TABLE 1.

TRANSFER OF PLASMIDS FROM *E. COLI* TO *ERWINIA AMYLOVORA*
STRAIN EA178

PLASMID	COMPATIBILITY GROUP	FREQUENCY OF TRANSFER PER DONOR CELL
F'_{LAC^+}	FI	5.0×10^{-8}
R100 <u>DRD</u> -56	FII	1.1×10^{-7}
R64 <u>DRD</u> -11	I	1.8×10^{-7}
RPI	P	1.4×10^{-3}
$E-LAC^+$?	2.0×10^{-7}

TABLE 2.

TRANSFER OF LAC^+ FROM *ERWINIA AMYLOVORA*
AND *ESCHERICHIA COLI* TO ENTEROBACTERIA

RECIPIENT BACTERIA	FREQUENCY OF LAC^+ TRANSFER (PER DONOR CELL) FROM	
	<i>E. AMYLOVORA</i> (EA178R2)	<i>E. COLI</i> (2395)
<i>E. AMYLOVORA</i> (EA178-S ₁)	1.0×10^{-4}	7.3×10^{-8}
<i>E. CHRYSANTHEMI</i> (EC16-S ₁)	1.2×10^{-6}	3.0×10^{-6}
<i>E. HERBICOLA</i> (Y46-S ₁)	1.0×10^{-6}	1.4×10^{-6}
<i>E. HERBICOLA</i> (Y74-S ₁)	1.1×10^{-5}	4.0×10^{-6}
<i>E. COLI</i> (2624)	3.1×10^{-2}	1.4×10^{-1}
<i>SHIGELLA DYSENTERIAE</i> (2880-S ₁)	2.3×10^{-1}	1.2×10^{-2}

TABLE 3.

EFFECT OF ACRIDINE ORANGE ON THE ELIMINATION OF LAC FROM THE
ERWINIA AMYLOVORA LAC⁺ HETEROGENOTE EA178R₁

ACRIDINE ORANGE CONC _N (μ G/ML)	GROWTH	NO. OF COLONIES EXAMINED		LAC ⁻ (% OF THE TOTAL NO. OF COLONIES)
		TOTAL	LAC ⁻	
0.0	+	2,376	18	0.75
0.2	+	1,960	87	4.4
1.0	+	1,409	1,254	89.0
2.0	NIL	NT	NT	NT

TABLE 4.

EFFECT OF ACRIDINE ORANGE ON THE ELIMINATION OF LAC FROM
ERWINIA AMYLOVORA PARENT STRAIN EA178R₁ AND DONOR
STRAIN EA178R₁-99

CULTURES	ACRIDINE ORANGE (μ G/ML)	TOTAL NO. OF COLONIES TESTED	NO. OF LAC ⁻ COLONIES	PERCENT LAC ⁻ OF THE TOTAL
EA178R ₁	0.0	1,147	7	0.6
	0.5	928	540	58.1
	1.0	844	804	95.2
EA178R ₁ -99	0.0	1,555	10	0.6
	0.5	1,428	9	0.6
	1.0	1,092	7	0.6

TABLE 5.

TIME OF ENTRY OF VARIOUS MARKERS IN INTERRUPTED MATINGS

DONOR	RECIPIENT	SELECTIVE MARKER	TIME OF ENTRY
HFR 99	BP 2158	SER ⁺	15'
		RIB ⁺	52'
		ILV ⁺	56'
HFR 159	BP 2158	SER ⁺	45'
		RIB ⁺	25'
		ILV ⁺	20'
		(His ⁺)	0'

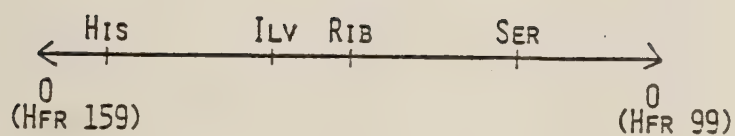


TABLE 6.

TRANSFER OF CHROMOSOMAL MARKERS FROM ERWINIA AMYLOVORA HFR 99
AND HFR 159 TO ERWINIA AMYLOVORA RECIPIENTS

RECIPIENT CULTURE	SELECTIVE MARKER	FREQUENCY OF TRANSFER (PER DONOR CELL) FROM	
		HFR 99	HFR 159
BP 2126	SER ⁺	2.2×10^{-4}	3.9×10^{-7}
	THR ⁺	2.0×10^{-7}	4.8×10^{-8}
	ILV ⁺	1.4×10^{-6}	3.8×10^{-4}
	LEU ⁺	1.5×10^{-6}	1.0×10^{-6}
M64S1-54	HIS ⁺	1.6×10^{-7}	1.7×10^{-5}
	TRP ⁺	3.1×10^{-7}	2.0×10^{-6}
M64S1-15	PRO ⁺	1.5×10^{-6}	1.0×10^{-4}
M64S1-68	ARG ⁺	6.1×10^{-6}	3.0×10^{-4}
BP 2152	ORN ⁺	3.7×10^{-6}	3.6×10^{-4}

TABLE 7.

VIRULENCE OF RECOMBINANTS FROM A CROSS
BETWEEN Hfr 99 AND BP 2157

Hfr 99: DERIVED FROM ERWINIA AMYLOVORA STRAIN EA178;
PROTOTROPHIC, STR^R, VIRULENT

BP 2157: SER THR ILV HIS RIB STR^R, AVIRULENT

SELECTED MARKER	OTHER MARKER INHERITED	VIRULENCE		
		# TESTED	# POSITIVE	% VIRULENT
SER ⁺	NONE	11	6	54.5
RIB ⁺	NONE	12	1	8.33
RIB ⁺	SER ⁺	12	3	25.0
RIB ⁺	ILV ⁺	12	1	8.33
ILV ⁺	RIB ⁺	10	NONE	0.0



FIGURE 1.

ISOLATION OF Hfr STRAINS IN ERWINIA AMYLOVORA EA178

EA178R1 (F'LAC⁺)GROW OVERNIGHT IN DARK IN THE PRESENCE OF
ACRIDINE ORANGE (2-4 μ G/ML)

PLATE ON EMB-LACTOSE AGAR PLATES

LAC⁺ CLONES (APPROX. 2 - 5% OF THE TOTAL POPULATION)

TRANSFER TO LURIA BROTH, LET GROW OVERNIGHT AT 30C

SPOT 0.02 ML ONTO THE LAWN OF RECIPIENT CELLS
(RECIPIENT CELLS PRESREAD ON SELECTIVE PLATES)

INCUBATE PLATES AT 30C

SPOTS WHICH PRODUCED NUMEROUS RECOMBINANT COLONIES ARE
LIKELY TO CONTAIN Hfr CELLS

"Transfer and acceptance of the R-factor RP1 by strains of *Erwinia herbicola* and *Erwinia stewartii*."

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Several strains of *Erwinia herbicola* (Lohnis) Dye and one strain of *E. stewartii* accepted the R-factor RP1 from *Pseudomonas aeruginosa* PA012r(RP1) by conjugation. The M.I.C. values for the antibiotics to which resistance determinants are carried by RP1 (i.e. carbenicillin, kanamycin, neomycin, tetracycline) were somewhat lower for the *E. herbicola* exconjugants than for the RP1 donor strain. Minor variations in some of the biochemical characteristics of the exconjugants, when compared with those of the parent recipient strains, were observed. The sensitivity of the RP1⁺ strains to RP1-specific bacteriophages PRD1 and PRR1 varied from an efficiency of plating (compared with *P. aeruginosa* PA067(RP1)) of zero to 133 for PRD1 on various *E. herbicola* exconjugant R⁺ strains, and from zero to 0.0002 for PRR1 on the same exconjugant strains. The corresponding values for the single strain of *E. stewartii* tested were 148 and 18.4, respectively. A phage-resistant strain, *E. herbicola* Y46(RP1), donated RP1 by conjugation to *E. herbicola* Y46 rif^r, *P. aeruginosa* PAT900, and *Escherichia coli* UB1005 only at very low frequencies, if at all. Transformation of *E. coli* JC7620 by covalently closed circular DNA from *E. herbicola* Y46(RP1) resulted in the acquisition of the RP1 antibiotic resistance pattern. The transformed strains were able to donate the R-factor to suitable recipients by conjugation. The results suggest that (i) some *E. herbicola* RP1 strains do not produce RP1 pili, or produce defective pili, and (ii) sensitivity to the RP1-specific bacteriophages is not a suitable means of detecting the presence of the R-factor RP1 in strains of *E. herbicola*.

This work was supported by the National Research Council of Canada, and will be published in detail in the Journal of Bacteriology (October or November issue, 1976).

Strain and Virulence Variability in Erwinia amylovora when
Exposed to E. amylovora Bacteriophage

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Soon after Burrill demonstrated that fire blight was caused by a bacterium, reports were published suggesting that variability existed among isolates of the pathogen, Erwinia amylovora (2,13,10,16). Ark was one of the first to perform an extensive study of this variability using 10 isolates (1). Since then other workers have attempted to better define this variability (4,9,15). Many factors such as temperature, host, nutrition, experimental technique, etc. have been suggested as cause of this variability. In the review of fire blight by Schroth et al. (14), it is pointed out that of the voluminous amount of literature published on fire blight surprisingly little information exists concerning the characterization of E. amylovora, especially the genetic variability of the pathogen in nature and how such diversity might relate to epidemiology.

This report presents some preliminary observations of a change in colony morphology and an attenuation in virulence of E. amylovora following exposure to bacteriophage. When a phage and its host interact one or more of the following events may occur:

1) the sensitive cells are eliminated and replaced with resistant mutants often of different potentialities; 2) destruction of the sensitive cells with conformation of the ability on the survivors to produce and transmit the phage to progeny (lysogeny); 3) alteration of genetic characterization of the survivors by transduction (6).

Normally when phage and its host are mixed, approximately 1 cell in 10^4 to 10^6 will survive (5). However when isolates of E. amylovora and phage PEal(h) were mixed the percent of survivors of the majority of isolates was found to be greater than 25% (Table 1) suggesting that the ability to survive the phage infection was due not to a mutation but to an adaptation. A mutation may be defined as a sudden inheritable change in the gene of an organism. Mutations occur spontaneously in bacteria at a rate of between 1×10^{-4} and 1×10^{-10} per bacterium per generation (5). An adaptation would involve a change not occurring until the selective agent was present. Proof of the occurrence of mutations in bacteria was not presented until 1943 when Luria and Delbruck reported their fluctuation test (12). The test was based on the principle that if resistant variants arose because of contact with the phage it should not matter whether populations exposed to phage came from a series of similar cultures or from one culture. However, if resistance occurred prior to exposure to the phage then the number of resistant variants among similar cultures would be different from that obtained from a series of samples from one

culture. The results obtained from performing such a test using E. amylovora #110 and #121 with phage PEal(h) as the selective agent are shown in Table 2. The variance among the individual cultures for #110 was less than the variance among samples from the single culture thus the variance was the result of sampling error. This adds support to the hypothesis that resistance of #110 to PEal(h) is due to adaptation rather than a mutation. With culture #121 the variance among individual samples was much greater than that among the samples from single cultures, suggesting that the resistant cells were derived from spontaneous mutations. These results suggest two different mechanisms involved in preventing lysis of the resistant or immuned cells. In #121 the mechanism is probably a change in adsorption sites on the cell wall, sufficient data is not available to explain the mechanism operating in #110 and similar isolates.

Colonies which developed from cells not lysed by PEal(h) exhibited a different colony morphology while in the presences of PEal(h). When E. amylovora wild type (w.t.) and phage resistant (p^r) isolates were grown on 2.0% nutrient agar supplemented with 0.5% glucose (NAG) for 36 to 48 hours, w.t. colonies were 2.0 to 2.5 mm in diameter, with an entire margin, convex, white, with a smooth, glossy surface. The p^r isolates when cultured under similar conditions, except in the presence of PEal(h), exhibited a colony morphology that was smaller, 1.0 to 1.5 mm in diameter, flat, with a translucent, grayish color and very easily distinguished from w.t. colonies. When colonies of the p^r type were cultured

in 0.8% nutrient broth, 0.5% glucose, and 0.25% yeast extract for 18 to 24 hours then plated on NAG without PEal(h) the colonies formed were of w.t. (Table 3). Furthermore, when agar was seeded with bacteria from these revertant types and PEal(h) was spotted on the agar surface typical areas of lysis occurred. However, when these p^r type colonies were transferred to NAG plates directly from NAG + PEal(h) a mixture of w.t. and p^r type colonies could be obtained. If this was done approximately four times relatively stable p^r type colonies occurred.

With many questions unanswered, the next step was to determine if the p^r type differed in virulence from the w.t. This was done using Jonathan apple seedlings. In two separate experiments (Fig. 1-A & B) the p^r type cultures showed an attenuation in virulence compared to w.t. cultures. All cultures were standardized to $\sim 2 \times 10^9$ colony forming units (CFU)/ml with $\sim 2 \times 10^7$ CFU applied to each seedling following wounding with a 25 gauge needle on a disposable syringe. The data in Figure 1-C were obtained from the same experiment shown in Figure 1-B with Figure 1-C showing the virulence rating for each individual isolate. Isolates 134 p^r and 121 p^r produced symptoms by 48 and 72 hours, respectively, while 105 p^r and 110 p^r did not produce symptoms during the 120 hours of the experiment. Isolations 134 and 121 also have higher mutation frequencies suggesting a different mechanism of phage resistance from that of the other isolates (Table 1). At 120 hours one replication of seedlings was sampled by crushing in 0.02 M potassium phosphate buffer and plated on

NAG + 50 µg/ml of rifampin (the bacteria contained a genetic marker). The results obtained are shown in Table 4. None of the seedlings inoculated with w.t. cultures exhibited p^r type colonies while seedlings inoculated with p^r type cultures produced p^r type colonies. Isolates 105 p^r and 110 p^r also produced w.t. colonies suggesting that reversion to w.t. may have occurred, this was not detected in 121 p^r or 134 p^r. It is also of interest to note that within the next 24 hours all seedlings were showing symptoms. There are several instances where phage are known to affect colony morphology or virulence of bacteria (3,7,8,11,17). A similar situation may be occurring with E. amylovora and its phage.

As mentioned at the beginning, much of this data is preliminary, some of the experiments have been repeated others are in the process or will be as well as explored in more detail. It appears that phage has an effect on the etiology of fire blight whether this ultimately affects the epidemiology of the disease is speculative at present.

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Table 1. "Mutation" frequencies of Erwinia amylovora isolates to the E. amylovora bacteriophage PEal(h).

<u>Erwinia amylovora</u> <u>isolate</u>	Mutation Frequencies(% Survivors)	
	<u>Experiment I</u>	<u>Experiment II</u>
105	79.4	15.4
110	63.8	37.2
112	26.8	48.1
113	57.8	37.5
115	73.9	84.8
116	72.1	
118	37.4	
119	36.0	
121	.00055	.00016
131		76.9
133		26.5
134		.0093
135		38.5
138		20.0

Table 4. Results of sampling seedlings inoculated with wild type (w.t.) and corresponding phage resistant(p^r) cultures of Erwinia amylovora. Sampling was done 120 hr after inoculation, all seedlings inoculated with w.t. cultures were showing symptoms while only seedlings inoculated with p^r cultures 121 and 134 were showing symptoms.

<u>Isolate</u>	<u>Number w.t. CFU</u>	<u>Number p^r CFU</u>
105 w.t.	400×10^7	0
105 p^r	26×10^7	47×10^7
110 w.t.	375×10^7	0
110 p^r	17×10^5	14×10^7
121 w.t.	75×10^7	0
121 p^r	0	125×10^7
134 w.t.	380×10^7	0
134 p^r	0	250×10^7

Table 2-(A and B). A) Results of fluctuation test with Erwinia amylovora isolate #110. B) Results using E. amylovora isolate #121.

A. Isolate #110.

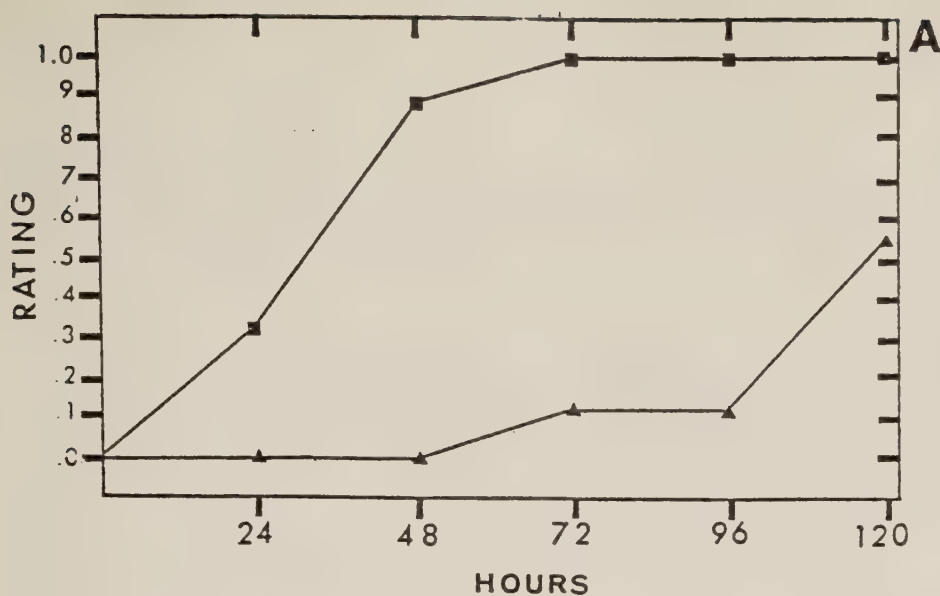
Samples from single culture		Samples from individual cultures	
Sample No.	No. resistant CFU	Sample No.	No. resistant CFU
1	29	1	25
2	29	2	23
3	32	3	29
4	45	4	42
5	40	5	18
		6	35
		7	19
		8	16
		9	22
		10	23
Average	35	Average	25
Variance	10.3	Variance	6.5
Chi-square	5.89	Chi-square	23.20
P	.273	P	.005

B. Isolate #121.

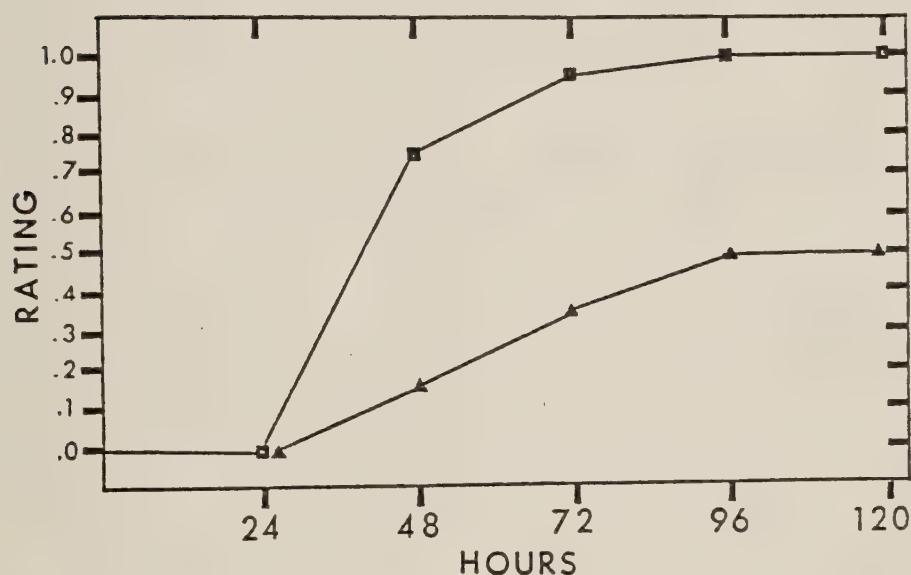
Samples from single culture		Samples from individual culture	
Sample No.	No. resistant CFU	Sample No.	No. resistant CFU
1	466	1	24
2	437	2	33
3	395	3	34
4	398	4	67
		5	35
		6	8
		7	250
		8	438
		9	15
		10	24
Average	424	Average	93
Variance	288	Variance	1974
Chi-square	8.14	Chi-square	1909.9
P	.052	P less than	.005

Table 3. Instability of phage resistant(p^r) *Erwinia amylovora*. Ten sub-cultures were made from #110 wild type(w.t.). These were plated on nutrient agar glucose(NAG) with phage PEa1(h), three colonies of phage resistant survivors were selected from the parent culture(p) and from each of three of the sub-cultures. Following 24 h incubation in nutrient broth glucose these were plated on NAG plates. The data shown are the results obtained from the parent culture, 110p, and from sub-cultures 110b, 110d, and 110f.

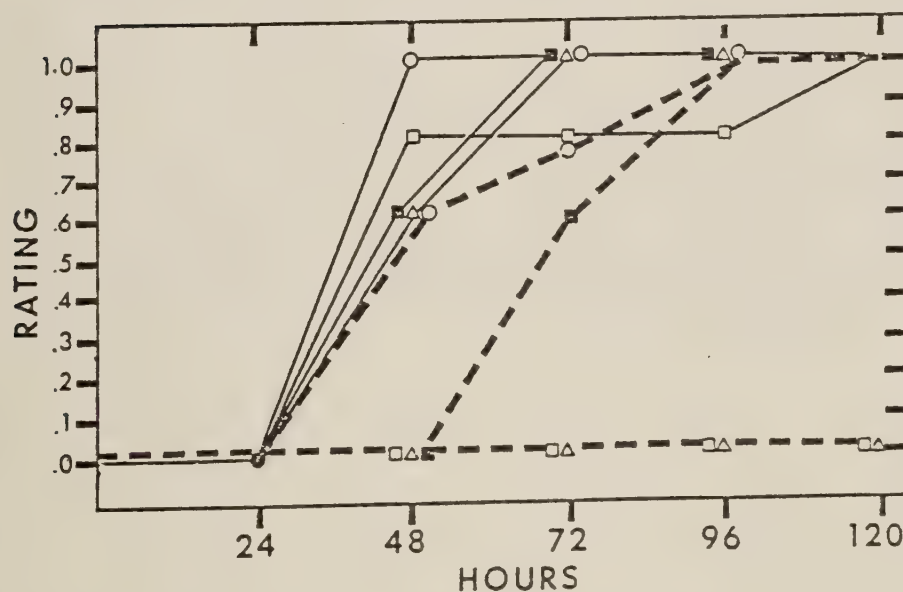
<u>Cultures</u>	<u>Growth on NAG + PEa1(h)</u>		<u>Growth on NAG</u>	
	<u>p^r CFU</u>	<u>w.t. CFU</u>	<u>p^r CFU</u>	<u>w.t. CFU</u>
110p w.t.	25 X 10^8	0	0	48 X 10^8
110p-1			0	29 X 10^8
110p-2			0	49 X 10^8
110p-3			0	21 X 10^8
110b w.t.	28 X 10^8	0	0	51 X 10^8
110b-1			0	58 X 10^8
110b-2			0	46 X 10^8
110b-3			0	56 X 10^8
110d w.t.	26 X 10^8	0	0	64 X 10^8
110d-1			0	49 X 10^8
110d-2			0	44 X 10^8
110d-3			0	31 X 10^8
110f w.t.	13 X 10^8	0	0	71 X 10^8
110f-1			0	77 X 10^8
110f-2			0	85 X 10^8
110f-3			0	90 X 10^8



Results using *E. amylovora* isolates 105, 115, and 121 wild type (■—■) and their phage resistant derivatives (▲—▲). Data are the results of the mean of the 3 isolates each consisting of 3 replications.



Results using *E. amylovora* isolates 105, 110, 121, 134 wild type (■—■) and their phage resistant derivatives (▲—▲). Data are the mean of the 4 isolates replicated 5 times.



Results obtained when the isolates used in B were plotted independently. Isolate 105 Δ, 110 □, 121 ■, 134 ○, wild types (—) and phage resistant derivatives (---).

Fig. 1-(A,B, and C). The results obtained when wild type and phage resistant [PEal(h)] *Erwinia amylovora* were inoculated into Jonathan apple seedlings. Virulence was determined by recording the time after inoculation of the first symptom expression; droplet of exudate at point of inoculation. Rating of 0 equals no disease while 1 equals disease. Seedlings were maintained at saturated relative humidity and a temperature of 23 to 25 C.

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OCCURRENCE OF FIRE BLIGHT ON RUBUS SPP. IN ILLINOIS. Stephen M. Ries, Alan G. Otterbacher, and Robert M. Skirvin, University of Illinois, Urbana, IL 61801

A bacterium was isolated from fruit mummies and blighted canes of several naturally-infected tetraploid thornless blackberry cultivars (Rubus hybrids) growing at Urbana, Illinois. Infected fruits were brownish, shriveled, hard, and remained attached to the spur. Yield was reduced on all cultivars; most seriously on selection SIUS 68-6-15 with 65.4% of the fruit infected. Infected canes were water-soaked, and purplish-black; the pith was necrotic, and bacterial ooze was present. Infection appeared to start at either the cane tips or at axillary buds and spread down the canes for distances of up to 20 cm. Pathogenicity was confirmed by isolation from infected plant parts and inoculating healthy blackberry cane tips. The host range of the blackberry bacterium included all publicly released cultivars of thornless blackberry as well as several numbered (unnamed) selections, five cultivars of Rubus allegheniensis; and three cultivars of R. idaeus. The bacterium did not infect 'Heritage' red raspberry, 'Jonathan' trees or 'Kieffer' pear fruit, but did produce limited water-soaking of green Jonathan fruit. E. amylovora isolates from apple and pear failed to infect Rubus spp. inoculated but produced typical fire blight symptoms on apple trees and abundant water-soaking and ooze on apple and pear fruit. Isolates of the blackberry bacterium and E. amylovora isolates were subjected to bacteriological tests, the methods used being those detailed and referenced in Bergey's Manual. Cells were single, straight rods, 0.5 by 1.0-3.0 μ m, motile by peritrichous flagella, Gram-negative, and were weakly facultatively anaerobic. All isolates required growth factors (nicotinic acid), liquefied gelatin, and were inhibited by KCN. All produced acetoin, mucoid growth on 5% sucrose nutrient agar and reducing compounds from sucrose. None of the isolates produced urease, indole, H_2S from cysteine, or gas from glucose. The isolates failed to hydrolyze casein, cotton seed oil or pectate, grow at 36 C, oxidize gluconate or reduce nitrate. The bacterium appears morphologically, biochemically and physiologically to be an E. amylovora isolate type which fails to infect apple trees. Starr et al. in 1951 isolated a similar bacterium from raspberry and named it Erwinia amylovora f. sp. rubi f. sp. nov.

Pertinent Statements Made During Discussion of the Causal Organism

Growth of E. amylovora in vitro:

maximum at 35-37°C

optimum at 23-30°C

minimum at 3-8°C

E. herbicola will not multiply at the same low temperatures which support the growth of E. amylovora; this should be taken into account in biological control. (Schroth)

A study of about 135 cultures of E. amylovora from numerous Rosaceous hosts and several countries with fire blight revealed that all isolates showed the characteristic pitting of the colony surface. (Goodman)

RESISTANCE

✓
ADVANCES IN RESISTANCE TO FIRE BLIGHT IN APPLES AND
PEARS: SCREENING METHODS, SOURCES, INHERITANCE
AND BREEDING — 1970-1976

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EVALUATION OF RESISTANCE

The announcement by Goodman et al (1974) that Erwinia amylovora produces a host-specific toxin, named amylovorin, was of great interest to apple and pear breeders. It raised the possibility of an easier method of evaluating resistance than by inoculation with the bacteria. The performance of amylovorin, however, has been disappointing so far. Although Huang and Goodman (1976) report observations suggesting that ultrastructural changes induced by amylovorin and E. amylovora are similar, Sjulín and Beer (1976) have evidence that amylovorin and E. amylovora induce shoot wilt by different mechanisms. Furthermore, Beer and Aldwinckle (1976) question the host-specificity of amylovorin, at least to apple. It should be emphasized that for amylovorin to be useful to breeders, it must 1) be conclusively demonstrated to be host-specific, and 2) be easier to use than the bacterium itself. Since these requirements have not yet been fulfilled, resistance studies are still being conducted with bacterial cell suspensions.

Pears

Oitto et al (1970) reported field observations of fire blight on 522 cultivars. Their data supplemented those of Reimer (1925) and many other early authors. An important sequel was the report by the same group of the results of artificial inoculation of some of the cultivars earlier recorded as resistant (van der Zwet & Oitto, 1972). When shoots were inoculated, many cultivars appeared less resistant than under natural infection. Several showed higher susceptibility when inoculated directly in the trunk than in the shoots. There was no relationship between percent blossom blight and degree of shoot blight.

Van der Zwet et al (1974) have added to our knowledge of sources of fire blight resistance in the genus Pyrus. They confirmed Reimer's (1925) report of great inter- and intra-specific variability.

Layne et al (1968) suggested that in some cases resistance is inherited quantitatively, in others qualitatively, regard-

less of the species source. They concluded that resistance was dominant in both cases. In contrast, J. M. Thompson et al (1975) hypothesized a dominant gene, Se, conditioning "sensitivity" or very high susceptibility to E. amylovora. In a sense this would mean that resistance is recessive. We believe that more data are needed before this hypothesis can be accepted fully. There were many significant deviations in Thompson et al's data, and the hypothesis only partly explains the data of Layne et al (1968).

The USDA group (van der Zwet & Oitto, 1973) and the workers at the Agriculture Canada Research Station, Harrow, Ontario (Layne et al, 1968) have published methods for screening pear seedlings for fire blight resistance. Although the greenhouse inoculations are quite simple and fast, evaluation is more time-consuming and growing the plants in the greenhouse for 4-6 months is most expensive in terms of time, space and labor. Nevertheless, these greenhouse methods are the most efficient yet available and a great improvement over observations of natural infection in the field. Significantly, the USDA and Harrow workers (Quamme et al, 1976) recently have shown that the greenhouse tests do, in fact, identify progenies which show resistance in the field.

No new fire blight-resistant pear cultivars have been introduced since Magness, Moonglow and Maxine were released in the early 1960's. Spartlet, a recent introduction from Michigan State University, is reported to be more "tolerant" of blight than Bartlett.

Pear Rootstocks

Westwood et al's report (1976) on the performance of Bartlett pear on Old Home X Farmingdale (OHxF) clonal rootstocks indicates that these stocks may be of great use to the pear industry. OHxF clones are resistant to fire blight (Reimer, 1950) and pear decline and condition a range of tree sizes.

Apples

There are many early reports on the susceptibility of apple cultivars based on field observations of natural infections. Recent, more comprehensive reports of this type include those of J. M. Thompson (1972) and Aldwinckle et al (1976). There are serious objections to such assessments, although for some cultivars they are all that is available. Variation in rootstock, tree age, nutrition, topography, soil type and bloom date all may obscure inherent differences between cultivars.

Shaw (1934) inoculated 25 apple cultivars artificially, but his cultivars are now mostly unknown and his data were not statistically analyzed. At Geneva, we have evaluated

most of the modern commercial apple cultivars by artificial inoculation of vegetative tissue (Aldwinckle & Preczewski, 1976). Replicated tests were conducted both in the greenhouse and the field. Trees were grown as single shoots and needle-inoculated in the apical meristem with 10^6 - 10^7 cells of E. amylovora. Lesion length and total shoot length were recorded when all lesions had ceased extending. Lesion lengths as a percentage of total shoot length were calculated (Table 1): the "percent lesion lengths" of cultivars in the field were correlated ($r = 0.56$; $P = 0.01$) with those of the same cultivars in the greenhouse. The relative susceptibility of well-known cultivars was generally in the same order that would be expected from field observations based on natural infection. The data for newer cultivars should be especially valuable since some of them had good fire blight resistance in addition to other desirable characters.

In contrast to pears, some commercial cultivars of apples do have a moderate and useful level of resistance to fire blight; e.g., Northwest Greening, Delicious, Empire, Winesap, Stayman (Aldwinckle & Preczewski, 1976). Gardner (1976) surveyed many species of Malus for fire blight resistance using artificial inoculation. He found a high level of resistance in many cultivars of Asiatic species, but in few cultivars of M. pumila. Intraspecific variation similar to that in Pyrus occurred. Gardner showed that two highly resistant crab apple clones, M. X robusta cv. Robusta 5 and M. X sublobata cv. PI 286613 transmitted their high level of resistance to a substantial proportion of their progenies.

Moore (1946) had suggested that three or more factors were involved in the inheritance of fire blight resistance in progenies from crosses among several commercial apple cultivars. Gardner (1976) made crosses among crab apple cultivars and rootstock clones, evaluated the progenies, and concluded that "genetic control of fire blight resistance in apple appears to be of at least two types, one based on the action of many genes and the other type conditioned by a few dominant genes". No evidence was found for a dominant gene for high susceptibility.

Fire blight resistance as a primary criterion in apple breeding is a new development. In fact, many apple cultivars introduced in recent years are unduly susceptible; e.g., Idared, Summerred, Tydeman's Early, Julyred, Virginiagold and Burgundy (Aldwinckle & Preczewski, 1976). In the last few years, fire blight resistance has been increasingly emphasized in breeding at Geneva. We have tried to adapt the methods used by the pear breeders, using named cultivars like Delicious as a source of moderate resistance. Since our apple seedlings are screened in the greenhouse when 2- and 12-weeks-old for resistance to apple scab and cedar apple rust, respectively, it is not feasible to screen for fire blight resistance the same year in the greenhouse. Screening the seedlings in the

following year after planting out in the field has proved inefficient because of the excessive care required to obtain uniform vigorous growth. This year, our best results have been by needle-inoculation of shoots of 3-year-old trees in the field.

Although no fire blight-resistant apple cultivars have been released as a result of deliberate breeding for resistance, a few recent introductions have proved to be resistant. They include cultivars from traditional quality-oriented programs (e.g., Britemac, Viking) and also Prima and Priscilla, products of the Purdue-Rutgers-Illinois scab resistance breeding program. Some of the Geneva scab-resistant selections (e.g., NY 55140-19, NY 55158-2) are similarly resistant to fire blight (Aldwinckle & Preczewski, 1976). In this case, the fire blight resistance is probably an added bonus transmitted fortuitously from the same source (Asiatic crab apple) as the scab resistance.

Apple Rootstocks

Interest in fire blight of apple rootstocks has arisen in recent years because of reports of dwarf trees on M.9 and M.26 rootstocks being killed by direct infection of the rootstocks (Cummins & Aldwinckle, 1974). Keil and van der Zwet (1975) reviewed the susceptibility of different rootstocks and also their effects on the susceptibility of scion cultivars grafted on them. There was general agreement on the susceptibility of the rootstocks, but conflicting reports on their effects on their effects on scions. M.26 has been reported both to increase and to decrease scion susceptibility. Increased scion susceptibility induced by MM.106, about which there is some agreement, involves more than just precocious flowering resulting in more infection courts. Scion physiology appears to be altered towards susceptibility. Further studies of rootstock influence on scion susceptibility will be made at Geneva in field plantings being developed specifically for this purpose.

The increasing incidence of fire blight on dwarfing rootstocks has forced us to consider fire blight resistance as a primary criterion in apple rootstock breeding. Sources of a high level of resistance in crab apple species can be utilized in this program immediately since fruit quality is unimportant. Gardner (1976) found that resistant seedlings of Robusta 5 and PI 286613 could be identified only 30 days after planting, when they had 4-6 leaves. This allows a much more efficient, screening of large numbers of seedlings, but is restricted to those families in which the high level of resistance occurs.

TABLE 1. Relative reaction of terminal shoots of field- and greenhouse-grown apple cultivars to artificial inoculation with Erwinia amylovora.

Cultivar	Field-grown			Cultivar	Greenhouse-grown		
	Cortical lesion length ^x				Cortical lesion length ^y		
	cm	%			cm	%	
York Imperial	42.3	100.0	a	Burgundy	40.2	95.2	A
Yellow Transparent	43.3	100.0	ab	Opalescent	28.8	83.7	AB
Idared	51.8	100.0	abc	Twenty Ounce	37.9	82.7	B
McIntosh	46.2	100.0	abc	Yellow Newtown	38.7	81.7	B
Milton	46.6	100.0	abc	Grimes Golden	24.7	77.7	BC
Niagara	43.8	100.0	abc	Rome Beauty	30.3	70.9	BCD
Opalescent	43.6	100.0	abc	Niagara	32.0	70.1	BCDE
Rhode Island Greening	44.8	100.0	abc	Idared	29.0	69.6	BCDE
Rome Beauty	39.7	100.0	abc	Tolman Sweet	18.0	68.3	BCDEF
Summerred	51.4	100.0	abc	York Imperial	32.0	67.6	BCDEF
Tolman Sweet	46.7	100.0	abc	Jenkins	19.3	64.4	CDEFG
Tydemans' Early Worcester	39.9	100.0	abc	Julyred	24.6	62.1	DEFG
Virgininiagold	53.8	100.0	abc	Monroe	28.4	59.5	DEFGH
Wagener	48.3	100.0	abc	Yorking	25.6	58.8	DEFGH
Wayne	53.6	100.0	abc	Jonagold	25.5	57.1	DEFGHI
Wealthy	42.3	100.0	abc	Jonnee	26.0	56.5	DEFGHI
Yorking	43.0	100.0	abcd	Barry	24.6	54.8	EFGHI
				Baldwin	19.0	51.4	FGHIJ

Honeygold	47.2	100.0	abcd	Yellow Transparent	24.7	51.2	FGHIJ
Mollie's Delicious	47.2	99.9	abcd	VPI 6	27.1	51.1	FGHIJ
Puritan	53.2	99.9	abcd	Lodi	23.0	50.5	FGHIJ
Yellow Newtown	41.5	99.8	abcd	Wagener	20.0	50.1	FGHIJ
Lodi	54.9	99.7	abcd	Tydemann's Early Worcester	20.4	49.1	FGHIJK
Twenty Ounce	46.1	99.6	abcd	Milton	24.3	48.8	FGHIJK
Macoun	33.6	99.5	abcd	Honeygold	17.3	48.4	FGHIJK
Burgundy	58.2	99.4	abcd	Jonamac	24.1	47.3	FGHIJK
Macspur	48.4	99.3	abcd	Webster	21.0	46.8	FGHIJKL
Jonathan	47.1	99.1	abcd	Nured McIntosh	24.8	46.7	FGHIJKL
Webster	43.4	98.9	abcd	Jonalicious	17.0	46.6	FGHIJKL
Julyred	40.4	98.7	abcd	NY 18491	15.0	46.4	FGHIJKL
Scotia	39.3	98.6	abcd	Macoun	14.3	45.8	GHijkl
Paulared	42.5	98.1	abcd	Magnolia Gold	23.3	44.9	GHijkl
Mutsu	48.8	97.4	abcde	Spigold	15.9	43.6	HIJKL
Grimes Golden	44.9	97.4	abcde	Spartan	16.0	42.3	HIJKL
Duchess of Oldenburg	33.9	97.0	abcde	Mutsu	17.3	40.1	IJKL
Jerseymac	49.2	97.0	abcdef	Ben Davis	16.3	39.7	IJKLM
Ben Davis	42.8	96.5	abcdef	Sungold	14.1	37.5	IJKLMN
Sungold	33.1	96.4	abcdef	Holly	19.8	37.2	IJKLMNO
Monroe	48.5	95.9	abcdef	Summerred	13.4	36.1	JKLMNO
Jonagold	31.5	95.5	abcdef	Jonadel	16.6	36.1	JKLMNO
Spigold	39.6	95.5	abcdef	McIntosh	16.7	35.7	JKLMNO
Nured McIntosh	34.5	95.3	abcdef	Scotia	12.6	35.7	JKLMNO

Magnolia Gold	45.8	94.9	abcdef	Turley	14.1	35.3	JKLMNO
Gravenstein	38.0	94.6	bcdef	Paragon	13.7	32.4	JKLMNOP
NY 58553-1	41.5	93.7	cdefg	NY 58553-1	15.9	32.3	JKLMNOP
Ring's Wealthy	39.5	93.5	cdefg	Jerseymac	10.5	32.2	JKLMNOP
Jenkins	37.9	92.1	cdefgh	Ozark Gold	14.1	32.0	KLMNOP
Horton Twenty Ounce	36.6	92.0	cdefghi	Wealthy	13.4	31.9	KLMNOP
Empire	39.3	91.2	cdefghi	Northern Spy	12.7	31.0	KLMNOP
Jonnee	36.9	91.1	cdefghi	Wayne	12.7	29.4	LMNOP
Jonamac	33.9	91.0	cdefghij	Cortland	11.8	28.3	LMNOP
Golden Delicious	39.8	90.7	cdefghij	Purdue 187-6	14.3	27.5	LMNOP
Spijon	37.0	90.6	cdefghij	Spijon	11.6	26.4	LMNOPQ
Early McIntosh	39.0	90.1	cdefghij	Mollie's Delicious	10.0	25.9	LMNOPQ
Holly	32.8	89.3	cdefghijk	Golden Delicious	11.0	24.0	MNOPQ
VPI 6	42.6	86.4	defghijk	Winesap	10.4	23.4	MNOPQR
Ozark Gold	45.0	84.2	efghijk	Melrose	12.2	23.1	MNOPQRS
Spartan	30.4	84.0	efghijk	Starkspur Golden Delicious	11.1	22.9	NOPQRS
Arkansas Black	28.1	83.9	efghijk	Primegold	9.6	21.5	NOPQRS
NY 18491	39.9	83.5	fghijk	Puritan	9.0	21.4	NOPQRS
Nured Rome	34.9	83.4	fghijk	Prima	9.7	20.6	OPQRS
Turley	34.9	82.7	fghijk	Early McIntosh	8.9	16.5	PQRS
Northern Spy	30.3	82.3	fghijkl	Delicious	8.8	16.3	PQRS
Paragon	41.1	78.6	ghijklm	Purdue 1279-2	7.2	14.6	PQRS
Delicious	36.0	77.7	hijklm	Empire	6.3	13.8	PQRS

Young America	29.1	77.1	hijklmn	Carroll	6.3	13.8	PQRS
Prima	30.8	76.1	hijklmn	Wellington	4.3	12.9	PQRS
NY 55140-9	34.6	76.0	hijklmn	NY 55140-19	4.0	12.6	QRS
Baldwin	34.1	75.3	ijklmn	Ring's Wealthy	5.3	12.4	QRS
Wellington	30.9	73.5	ijklmn	Hawaii	5.5	11.8	QRS
Melrose	34.7	72.2	jklmn	NY 55158-2	5.1	10.9	RS
Vermont Spur Delicious	23.8	70.9	jklmn	Northwestern Greening	3.9	9.4	RST
Starkspur Golden Delicious	32.1	68.9	klmno	Quinte	3.5	9.2	RST
Carroll	27.4	60.9	lmnop	Starkrimson Delicious	3.3	8.4	RST
Priscilla	25.0	60.4	lmnop	Crimson Beauty	2.3	8.2	RST
Winesap	22.3	57.1	mnpq	Priscilla	3.7	8.0	ST
Stayman	25.0	56.5	nopq	Viking	3.0	7.3	ST
Viking	18.1	47.3	opqr	Britemac	1.0	1.8	T
NY 55140-19	22.2	44.2	pqr	Ottawa 523	1.6	1.8	T
Starkrimson Delicious	12.1	42.7	pqrs				
Dolgo	21.6	38.7	pqrs				
Jonadel	17.8	32.5	qrs				
Primegold	14.4	32.4	qrst				
Northwestern Greening	15.6	31.7	rst				
Hawaii	16.9	29.2	rst				
Jonalicious	11.7	28.8	rst				
Quinte	12.9	22.8	stu				
Purdue 1279-2	12.1	19.7	stu				

Crimson Beauty	6.5	11.3	tuv
Britemac	10.9	9.7	uv
NY 55158-2	6.7	8.8	uv
Ottawa 523	7.3	5.5	v
Purdue 187-6	3.9	4.4	v

x Means of 15 replicates of each cultivar. % = lesion length (cm)/total shoot length (cm) x 100. Percent lesion lengths followed by same letter did not differ significantly ($\underline{P} = 0.05$) according to Duncan's multiple range test on arc-sin transformed data.

y Means of 7 replicates of each cultivar. % = lesion length (cm)/total shoot length (cm) x 100. Percent lesion lengths followed by same letter did not differ significantly ($\underline{P} = 0.05$) according to Duncan's multiple range test on arc-sin transformed data.

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CAN AMYLOVORIN BE USED TO SCREEN APPLE CULTIVARS
FOR RESISTANCE TO ERWINIA AMYLOVORA?

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Goodman, Huang & Huang [Science 183:1081-1082 (1974)] described the isolation of a host-specific phytotoxin (amylovorin) from fire blight ooze which caused wilt of excised shoots of rosaceous plants. They suggested that it might be used in pome fruit breeding programs to screen selections for resistance to Erwinia amylovora rapidly. Recent work by Sjulín and Beer [Proc. Am. Phytopathol. Soc. 2:107 (1976) and 1976 APS Meeting Abstract] has indicated that amylovorin induces wilt of excised shoots by causing water deficits. More recently, Goodman and coworkers [1976 APS Meeting Abstracts] have presented histological evidence that xylem vessel plugging occurs in amylovorin-treated shoots. The primary purpose of the present work was to determine if, among apple cultivars, susceptibility to wilt induced by amylovorin is correlated with susceptibility to E. amylovorin to a degree sufficient to permit its use in screening for resistance.

We used plants of nine Malus pumila cultivars that differed in fire blight susceptibility that had been grown in the greenhouse and were available in large numbers. The susceptibility of the cultivars to E. amylovora had been evaluated previously by Aldwinckle and Preczewski [Proc. Am. Phytopathol. Soc. 2:94 (1976) and Phytopathology, in press] by artificial inoculation of similar trees in the greenhouse and field nursery. Susceptibility was based on percent of new shoot length that became infected before cankers were formed.

Plants were grown in 5-inch (12.7 cm) plastic pots in a mixture of peat, perlite and vermiculite (1:1:1) on M.7 rootstocks. All plants were cut back to a single bud and grown as single shoots for 3 weeks prior to the amylovorin test.

Preliminary experiments with apple shoots and extensive work with Cotoneaster pannosa shoots by Sjulín and Beer (unpublished) had indicated that shoot flexibility was correlated with susceptibility of excised shoots to wilt in amylovorin solutions. To take this factor into account, the relative force required to displace each intact shoot apex a given distance was determined.

After measuring shoot length and flexibility, the apical 10 cm of each shoot was excised, recut under water to 8 cm, placed in beakers of tap water and held in the test chamber overnight. Immediately before treatment with amylovorin, shoots were recut again under water to 6 ± 0.5 cm length, measured from the meristem to the shoot base.

Two samples of amylovorin were used. The first, kindly provided by R. N. Goodman in August, 1976, and designated "RNG" had been purified from fire blight ooze produced on immature Jonathan fruits. The second preparation, designated "TMS", had been purified by T. M. Sjulín from ooze produced on immature Pyrus communis 'Bartlett' fruits in 1975 by slight

modification of procedures published by Goodman et al. Both preparations were diluted to 100 μ gm per ml with sterile distilled water, stirred magnetically for 0.5 hr and then dispensed into 10-ml vials supported in polystyrene foam racks.

Four shoots of each cultivar were treated with each of the two amylovorin preparations; the whole experiment was repeated. Therefore a total of 16 shoots of each of the nine cultivars was exposed to amylovorin. Similarly treated shoots of each cultivar that were incubated in sterile distilled water served as controls.

Amylovorin sensitivity tests were conducted under controlled-environment conditions because previous studies indicated that environmental conditions drastically affected the reaction of excised roseaceous shoots to amylovorin solutions. Our tests were conducted in a walk-in chamber at 24 ± 0.5 C, 70-80% relative humidity, and 19.4 klux light provided by a combination of incandescent and fluorescent lamps. At hourly intervals, the leaf and shoot wilt was evaluated. Each shoot was assigned an integer rating on the following scale. 0 = complete turgidity; 1 = flaccid or wilted leaf lamina; 2 = wilted, shoot bent 0-30° from the vertical; 3 = shoot bent 30-60°; 4 = shoot bent 60-90°; and 5 = shoot bent more than 90° from vertical. During the experiments, additional test solutions were added to vials as needed.

Results and Discussion

Most shoots of most cultivars had wilted after 7 hrs exposure to amylovorin. The first evidence of water deficit usually occurred after 5 or 6 hrs. Both preparations of amylovorin induced wilt of shoots of all cultivars but cultivars varied in their mean wilt index ratings. None of the shoots continuously incubated in water exhibited wilt symptoms. The RNG amylovorin induced wilt to a greater extent than the TMS amylovorin. This may have been due to suspended particulates since that preparation appeared cloudy.

The mean wilt indices of shoots of the nine cultivars treated with the RNG amylovorin preparation were slightly higher than the TMS means. However, there were only small differences in the ranking of the cultivars by the two preparations on the two test days. The mean wilt index of shoots of the nine cultivars in both amylovorin preparations on both test days are presented in Table 1. The flexibilities of shoots among cultivars did not differ significantly although flexibility was correlated with shoot length. The cultivars, arranged on the basis of mean wilt index fall into statistically distinct groups. Shoots of Turley, Rome Beauty, Arkansas Black and Prima wilted to a significantly greater extent than did shoots of Ben Davis, McIntosh, Tolman Sweet and York Imperial.

The crucial question is whether cultivar response to amylovorin has any relationship with the response of the same cultivars to E. amylovora. For susceptibility to E. amylovora, we relied on Aldwinckle and Preczewski's previous data. Under quite uniform conditions in both the field nursery and in the greenhouse, plants comparable to those that were tested for

amylovorin sensitivity, were inoculated artificially. The same 9 cultivars are ranked in descending order of susceptibility to E. amylovora in Table 1. The figures represent the percent of the current year's shoot that became infected in the greenhouse test. The ranking of the same cultivars in the field test was highly significantly correlated with the greenhouse test rankings. (Arkansas Black was not included in the greenhouse test and it is ranked for susceptibility based on a field nursery test that included all the cultivars.) These rankings agree well with the observations of others that indicate that York, Rome and Tolman are among the more susceptible cultivars, whereas Prima, Arkansas Black and Golden Delicious are less susceptible. Ben Davis, McIntosh and Turley are intermediate in susceptibility.

A plot of mean wilt index data vs. percent shoot blight data for the cultivars is depicted in Figure 1. Linear regression analysis of the wilt index and E. amylovora susceptibility data indicated no significant relationship ($r = -0.365$). When we compared the rankings of the 9 cultivars based on susceptibility to E. amylovora and sensitivity to amylovorin-induced wilt, by Spearman's Rank Correlation test, (Snedecor and Cochran, 1967) again there was no significant relationship ($r_s = -0.333$). In this test, for significance at $P = 0.05$, r_s must be $\geq +0.683$.

If the nine cultivars which we tested with amylovorin were of unknown susceptibility to E. amylovora, we would have considered Prima and Turley as susceptible, and York and Tolman as resistant, when actually the relationships are reversed.

The results of these studies indicate that cultivars differ in their sensitivity to amylovorin. But, unfortunately amylovorin sensitivity of the nine apple cultivars tested was not correlated with susceptibility to E. amylovora. Therefore, we feel that amylovorin cannot be used to rank cultivars for fire blight susceptibility. In addition, our findings do not support the supposition that amylovorin is a host-specific phytotoxin.

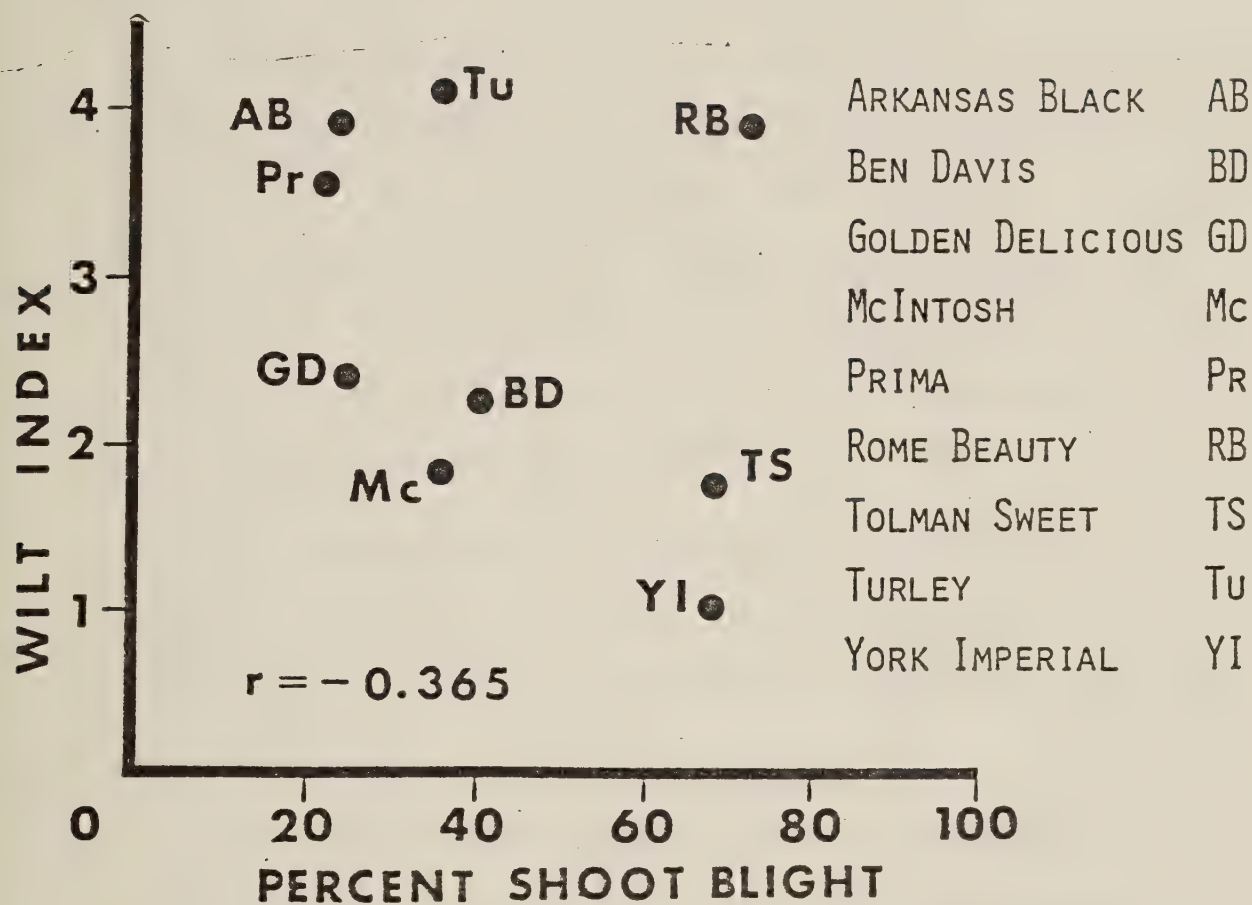
Table 1. Fire Blight Susceptibility - Amylovorin Sensitivity

<u>Cultivar</u>	<u>% Blight</u> ¹	<u>Cultivar</u>	<u>Wilt Index</u> ¹
Rome Beauty	71 a	Turley	4.13 a
Tolman Sweet	68 a	Rome Beauty	3.94 ab
York Imperial	67 a	Arkansas Black	3.89 abc
Ben Davis	40 b	Prima	3.56 abc
McIntosh	36 bc	Golden Delicious	2.31 bcd
Turley	35 bc	Ben Davis	2.25 cd
Golden Delicious	24 bc	McIntosh	1.81 d
Arkansas Black ²	-- --	Tolman Sweet	1.75 d
Prima	21 c	York Imperial	1.00 d

¹ Within columns, data followed by the same letter do not differ significantly ($P = 0.05$).

² Ranked here on the basis of another test.

Figure 1.



Rootstock susceptibilityEve Billing^{1/}

The following methods have been used to test the fireblight susceptibility of apple rootstocks: inoculation of the stem, a petiole or a cut leaf just above the last unfolded leaf. Infection without obvious damage has been achieved by wetting shoots before dipping tips into a suspension of the pathogen. Petiole inoculation is now the standard procedure.

M26 proved highly susceptible regardless of the method of inoculation. With some low susceptibility stocks, the wet method produced more infections than the cut leaf method.

Preliminary experiments were made this year on the effect of the pre-inoculation growth regime on the susceptibility of four apple rootstocks. It seemed that the susceptibility of some rootstocks was higher under a high temperature than under a low temperature regime.

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POSSIBILITY OF COMBINING LOW LEVELS OF FIRE BLIGHT RESISTANCE
IN PEAR

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Abstract

Low levels of fire blight resistance within *P. communis* can be successfully combined and increased to a height not observed previously in this species. Combinations of 'Bartlett', 'Seckel', 'Comice', and 'Ree Carlo di Wurtemberg' (RCW) each provided some degree of resistance which were additive, resulting in resistant selections such as Mich.-US 437, US 301, US 307, US 309, US 342, US 539, and others. The combination of additive sources of resistance have resulted in highly resistant cultivars such as Magness, Moonglow, and Dawn.

Resistance to fire blight can be destroyed, however, by combining resistant selections with cultivars such as 'Conference', which contribute susceptibility to progeny. Evidence has been obtained for a dominant gene, *Se*, in these cultivars, causing unusual sensitivity to the blight organism. Approximately half of the variability in resistance to blight in pear is additive. The high frequency of highly resistant selections resulting from crosses between nonsensitive (*sese*) *P. communis* cultivars indicates that selection for high resistance within progenies of such populations will result in a genetic gain.

Introduction

Most cultivars of the dessert pear (*P y r u s c o m m u n i s*) are susceptible to fire blight but degrees of susceptibility may range from moderate to very susceptible. High degrees of resistance have been observed only in other *P y r u s* species and their hybrids. Using species other than *P. c o m m u n i s* in breeding has the disadvantage that fruit characteristics of the offspring are not readily accepted commercially and it will take several generations to reach the desired size and quality. Blight data accumulated in our pear breeding program indicated that a considerable number of seedlings in progenies of low to moderately resistant parents were resistant. A brief discussion on possibilities to develop blight resistance from relatively susceptible material is presented in this report.

Materials and Methods

We have produced numerous hybrid progenies in the pear breeding program. The seedlings were planted in the field spaced 120 cm. apart in 90 cm double rows, 4.8 m apart. Trees were maintained in clean rows in sod and fertilized early each spring and again in early summer so that tree growth was vigorous and very succulent.

Fire blight occurred as natural infection and no attempt was made to prevent or control it by pruning or spraying. All trees in this orchard were subjected to several blight epiphytotics. Each fall, individual trees were scored for total amount of blight damage. Scores, based mainly on age of wood infected and total

percent of tree blighted, were designated by a numerical code from 10 to 1 with the higher scores indicating the least damage (van der Zwet et al., 1970). Blight class 10-8 is considered highly resistant, 7-6 resistant, 5 susceptible, and 4-1 very susceptible. Bell et al. (1976) established on the same material the adjusted phenotypic score (APS) by indirect comparison of parents common to each evaluation. They also computed the average combining ability (ACA) from mean progeny fire blight scores as a measure of parental prepotency for transmission of resistance. Each calculation involved 227 progenies.

Sensitivity genotypes Sese or sese and the tendency to produce an excess of sensitive seedlings (TSS) were determined by Thompson et al. (1975) from genetic analysis of the above material.

For the purpose of this discussion, only selected examples are used to illustrate the point. Detailed information can be obtained from the original papers.

Results and discussion

The selected examples presented in Figure 1 illustrate that there are additive and non-additive effects in fire blight resistance. Crosses of parents with relatively low fire blight scores (Fig. 1A) resulted in phenotypic selections which, when further crossed with either 'Comice' or 'Ree Carlo di Wurtemberg' gave resistant selections and a high percentage of resistant seedlings in subsequent progenies. This illustrates the additive nature of fire blight resistance in certain parents.

In contrast, all crosses where 'Conference' was used as a parent resulted in susceptible seedlings and this cultivar behaved as a non-additive parent transmitting a high degree of susceptibility (Fig. 1C).

There are intermediate cases between the two. In almost all progenies, the original cross was made with 'Bartlett' resulting in relatively resistant selections, but when 'Bartlett' was used again, the proportion of resistant seedlings in the progeny decreased to very low levels (Fig. 1B). This character of 'Bartlett' will be discussed later.

All 6 progenies in this study contained the susceptible cultivar Barseck ('Bartlett' x 'Seckel') three generations back as the maternal or paternal line of the cross. In turn, 'Barseck' x 'Bartlett' resulted in the moderately resistant selection Mich.-US 437, which has been extensively used in our breeding program. Crossed with the cultivars 'Comice' and 'RCW', it has produced several resistant selections like US 301, 307, 309, 342, 539, and others. The selections US 353, US 570, and US 3866-E were released in 1960 as cultivars 'Moonglow' (Comice x RCW), 'Dawn' (Mich. US 437 x Comice), and 'Magness' (Seckel seedling x Comice), respectively.

The genetic characteristics of the parents, used in Figure 1 are given in Table 1. Characterization about the presence or absence of the Sese gene, the adjusted phenotypic score (APS) and the average combining ability (ACA) are valuable when parent

selection is important. Information on the tendency to produce excessive sensitive seedlings (TSS) is available for only a few of the parents, but it could aid in further characterizing parents with the Sese genotype. The data in Table 1 indicate that the combination of low level of resistance is most successful if the genotypes are sese or Sese with a 0 TSS. When the TSS score is one plus, as in the case of 'Bartlett', the combination is not nearly as successful. When a cultivar with the Sese genotype produces an excessive quantity of sensitive seedlings, resistance is completely destroyed in the progeny.

It appears that, based on several hundred crosses in our breeding program, there are four independent sources of low levels of blight resistance. These sources are 'Seckel', 'RCW', 'Comice', and 'Bartlett'. 'RCW' may be considered as a moderate to high source of resistance, whereas 'Bartlett' is a very low source.

Evaluation of the genetic inheritance pattern of each of the four cultivars transmitting blight resistance is difficult. Several research workers have tried to evaluate combining ability to produce resistant seedlings. Their methods differed mostly because of the disagreement on the classification of phenotypic resistance of the cultivar studied. In a review of pear cultivars, 'Seckel', was cited by 6 authors as resistant, 7 as susceptible, and 15 as moderately resistant (van der Zwet and Keil, 1977). Bell et al. (1976) and Thompson et al. (1975) indicated that it carries the Sese gene with a 0 TSS. In our experience, 'Seckel'

is a parent of the most resistant group of seedlings and selections. Therefore, we rated this cultivar as at least moderately resistant to fire blight.

'RCW' is very similar with the exception that less data is available on this cultivar. It behaved in our breeding program the same as Seckel and numerous artificial inoculations produced negative results (van der Zwet, 1976). Therefore, we regard 'RCW' as a very good source of fire blight resistance.

'Comice' was one of the few cultivars in the resistant group with an average score of 7.0 in a severely blighted orchard at Beltsville (van der Zwet et al., 1974). Even though one tree died from blight, 57% of the trees were moderately resistant. Thompson et al. (1975) rated 'Comice' as sensitive to fire blight phenotypically and to be heterozygous at the Se locus. In our breeding program, 'Comice' has produced a considerable number of resistant selections.

'Bartlett' is usually regarded as very susceptible. According to Bell et al. (1976), it has an APS rating of 2.3 and carries the Sese genes. In some of the early crosses, it produced relatively resistant selections. 'Bartlett' was also regarded having an ACA of 4.0. We know from experience that it often takes several years for 'Bartlett' trees to die following infection. This is in contrast to trees of cultivars that can be destroyed by blight within one year. This apparent resistance may be associated with the resistance of the trunk and major scaffold limbs to the blight

organism (van der Zwet and Oitto, 1972). However, it could also be due to some type of general resistance. The resistance evaluation of 'Bartlett' is confused because, when 'Bartlett' is used to improve fruit quality at late stages of crosses, it destroys resistance, but when Laxtons Progress ('Marie Louise' X 'Bartlett') is used, resistance is apparently maintained. Therefore, 'Bartlett' may carry a low level of resistance but its Se gene may be diluted before expressing its resistant character.

'Conference' is an excellent example of a susceptible cultivar. It is much more susceptible than 'Bartlett'. When 'Conference' becomes infected by blight, the tree is destroyed by blight within a short period of time. Bell et al. (1976) gave 'Conference' an APS of 1.1. Thompson et al. (1975) indicated that an excessive number of sensitive seedlings were produced in its progenies. On this basis, we have concluded that it should be excluded from breeding programs which require a reasonable level of resistance. Similar cultivars include 'Ananas de Courtrai', 'Devoe', and 'Forelle' (van der Zwet and Oitto, 1972; van der Zwet et al., 1974*).

We have developed fire blight resistance within *P. communis* by combining low levels of resistance and by avoiding Sese genotypes with a tendency to produce an excess number of sensitive seedlings. Species crosses have no advantage if the only purpose is the development of fire blight resistance. Species crosses have an advantage over *P. communis* crosses when, insect or fungus resistance is required along with fire blight resistance, since those two characteristics are not present in *P. communis*.

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A**MAGNESS**

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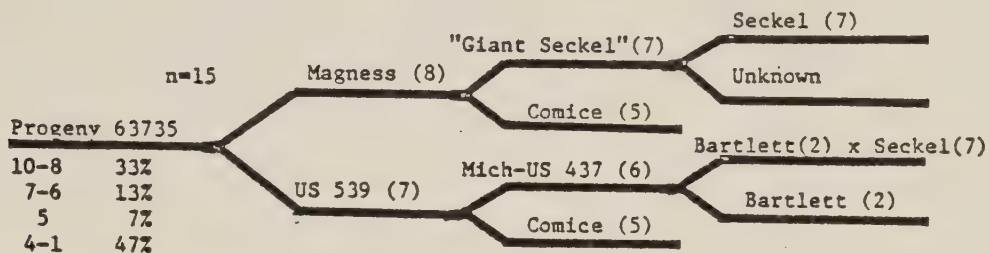
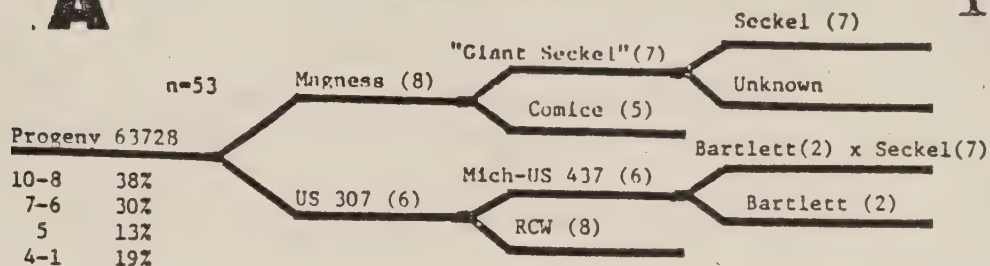
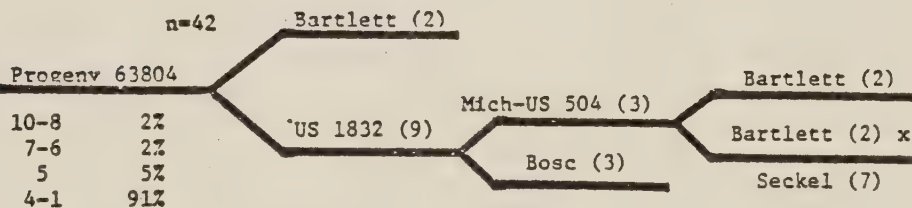
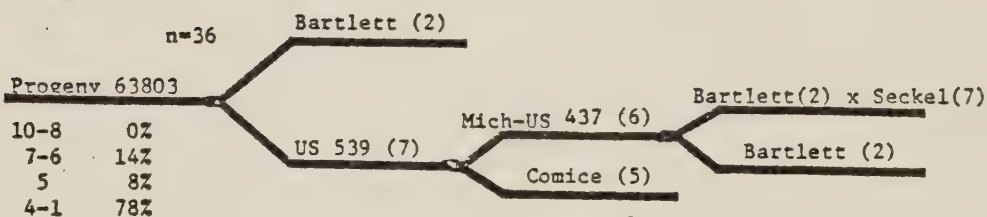
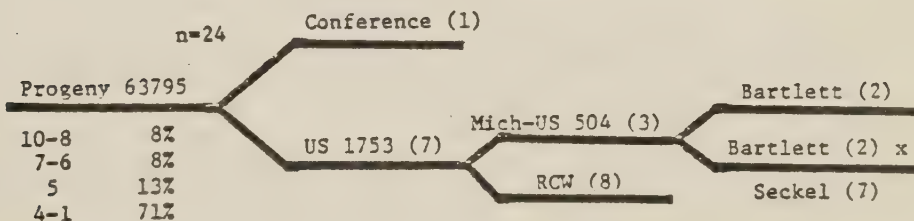
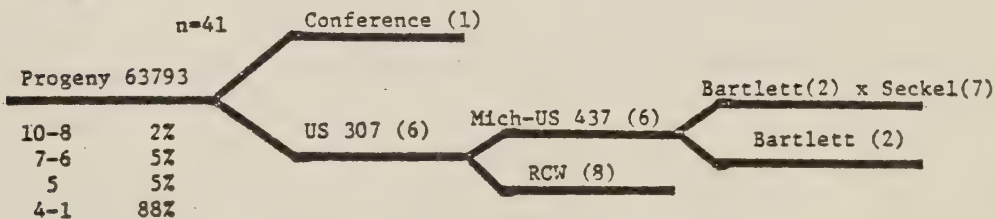
**B****BARTLETT****C****CONFERENCE**

Figure 1. Examples of combinations of low levels of fire blight resistance in seedling progenies within *Pyrus communis* (Blight scores are in parenthesis.)

Table 1. Summary of genetic characteristics of certain pear cultivars and selections (after Bell et al., 1976 and Thompson et al., 1975).

Cultivar/ selection	Sensitivity genotype	APS <u>1</u> /	ACA <u>2</u> /	TSS ³ /
US 1832	sese	8.6	4.4	
Magness	sese	8.3	5.2	
RCW		8.0 <u>4</u> /		
US 301	sese	7.1	5.3	
US 1753	sese	7.1	6.3	
Giant Seckel		7.0 <u>5</u> /		
Seckel	Sese	7.0 <u>4</u> /	4.1	0
US 539	sese	6.8	4.3	
US 307	sese	6.7	4.8	
Mich-US 437		6.1		
Comice	Sese	4.7	3.8	0
Mich-US 504		2.7		
Beurre Bosc	Sese	2.6	3.8	+
Bartlett	Sese	2.3	4.0	+
Conference	Sese	1.1	3.1	++

1/ Adjusted phenotypic score

2/ Average combining ability

3/ Tendency to produce excess of sensitive seedlings

4/ Modified score from Bell's original calculated figures of 1.8 (RCW) and 1.9 (Seckel)

5/ Lowest blight score recorded at Beltsville

Pertinent Statements Made During Discussion on Resistance

Penetration of E. amylovora through leaf glands occurs most frequent when the leaf is extremely young. As the leaf ages, the glands become less susceptible. (Goodman)

Petiole inoculation - Inoculation through petiole of leaf close to the last expanded leaf. (Billing)

Stem apex inoculation - There is no such thing as a stem apex; just a bunch of leaves that have petioles. (Aldwinckle)

TOUR OF SOME FIRE BLIGHT FIELD EXPERIMENTS
AT GENEVA EXPERIMENT STATION, SEPTEMBER 22, 1976

J. L. Preczewski and Herb S. Aldwinckle

Comparative field screening of 1- and 3-year-old apple seedlings for susceptibility to fire blight

The purpose of this study is to develop a field method of determining vegetative fire blight susceptibility to assist in the breeding program for developing resistant varieties. In the past, a midsummer fire blight screening test was done in the greenhouse after the seedlings had been screened for scab and rust susceptibility. The need for more uniform plant growth influenced the decision to conduct the fire blight screening test after the seedlings had been planted in the field.

Several thousand 1- and 3-year-old seedlings planted as 1-0 stock in seedling rows were needle-inoculated in mid- to late July. Early results indicate that testing 3-year-old seedlings is the preferred method because of the reduced need for pest control and the production of multiple readings per seedling. Sample progenies are shown in Fig. 1.

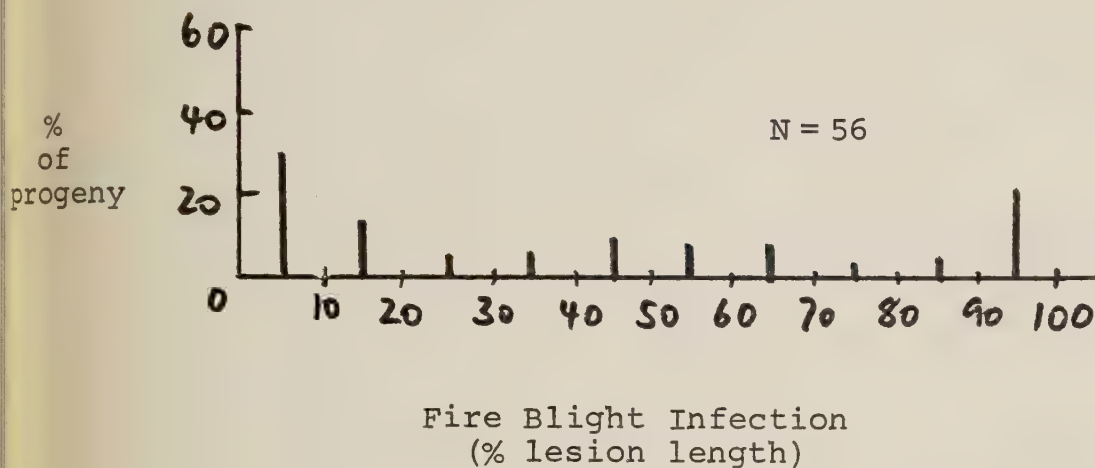
Establishment of an apple orchard suited for field evaluation of fire blight control measures

The primary purpose of this orchard is to carry on the epidemiological and control studies begun at Epicon I in Wayne County. The orchard is well established, and beginning in 1977 techniques perfected at Epicon I will be used in applying and evaluating treatments. The orchard, which is located on the Vegetable Research Farm, consists of two sections. The first section, which will be used to test blossom blight control measures, consists of 90 3-tree plots; every plot containing one tree each of the cultivars Empire, Idared and Wayne. These cultivars are representative of early-blooming and late-blooming susceptible varieties and a resistant variety. The other orchard section, which will be used to test vegetative blight control measures, consists of 77 3-tree plots of the susceptible variety Idared.

Establishment of an apple orchard suited for field studies on varietal resistance

One purpose of this orchard is to evaluate fire blight blossom susceptibility of 35 economically important or promising varieties and numbered selections. The planting consists of 8 single tree reps of 33 varieties and 35 single tree reps of two reference varieties; Idared and Monroe. In 1978, trees growing in this orchard will be subjected to a controlled inoculation by methods being developed at Epicon I. This orchard is located adjacent to the control evaluation orchard (see map).

Cross 72742: NY 53710-95 X NY 55140-19
(susceptible) (highly resistant)



Cross 72700: Ottawa 523 X NY 55140-19
(highly resistant) (highly resistant)

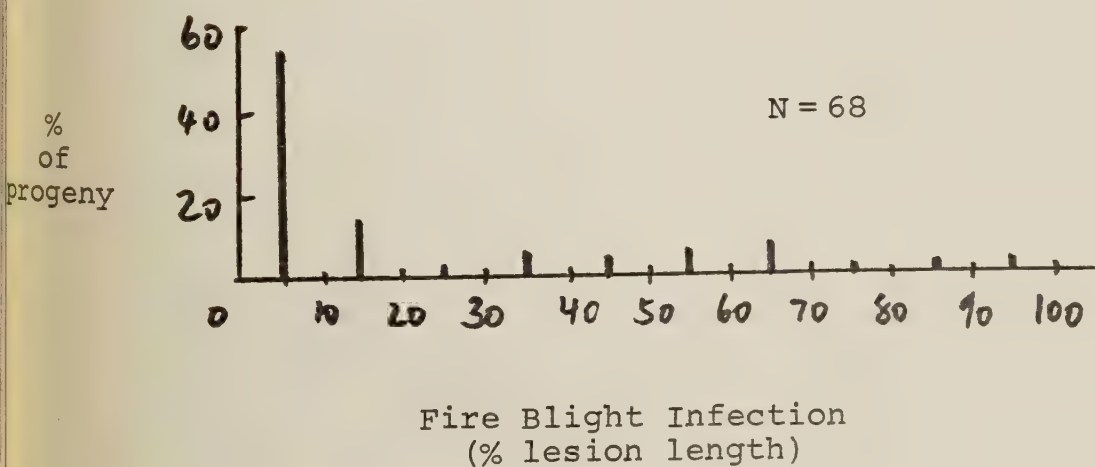
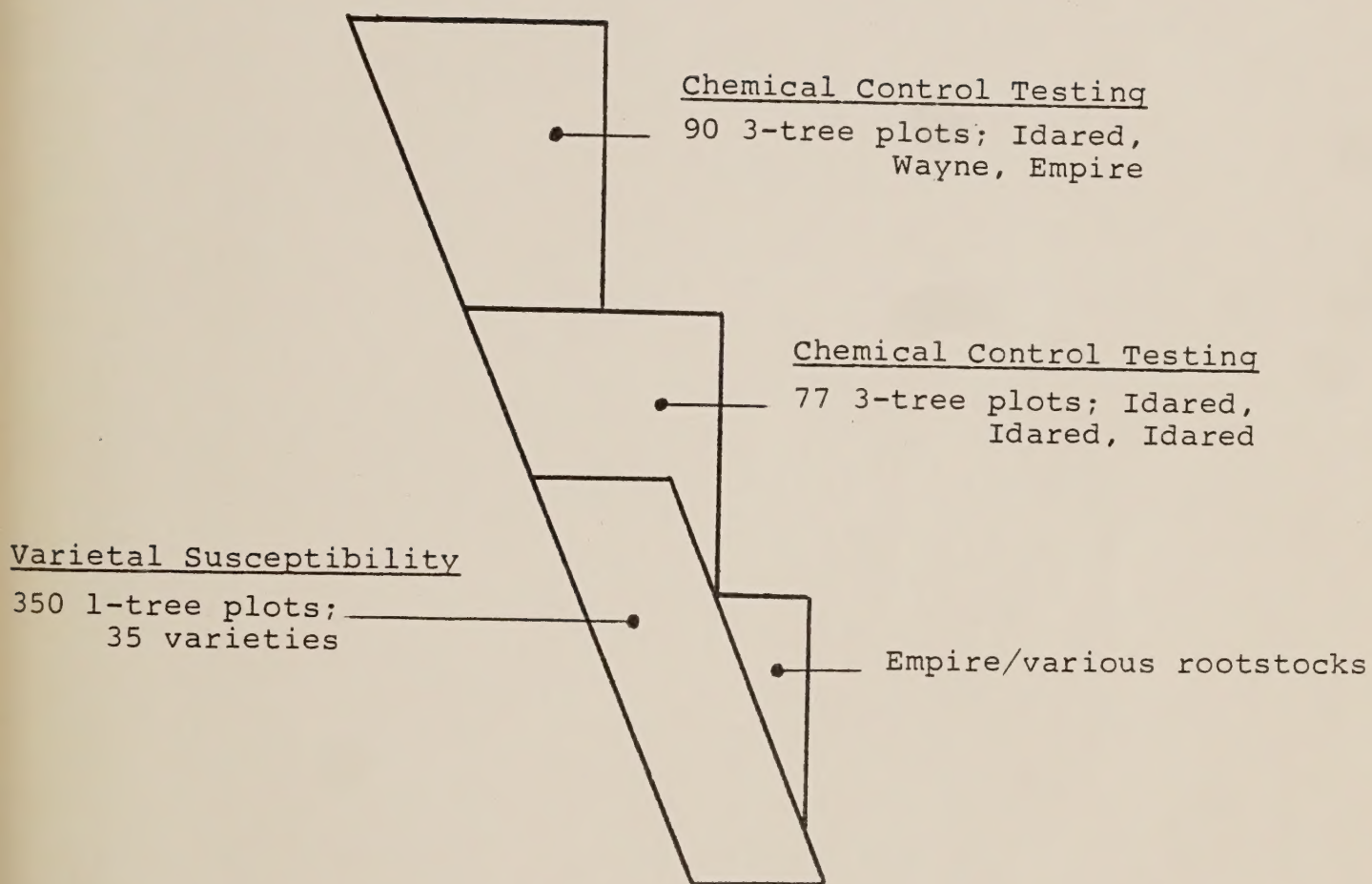


Fig. 1. Reaction of sample progenies to inoculation with Erwinia amylovora as 3-year-old seedlings in the field.

Fire Blight Orchards - Vegetable Research Farm

GATE

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Total planting - 850 trees
Planted in spring, 1975; 0-2 stock
Malling 7 rootstock
10' x 17' spacing; 15' between plots
Total area - 4.2 acres

